Potential Corticoid Metabolites: Chemical Synthesis of 3- and 21-Monosulfates and Their Double-Conjugates of Tetrahydrocorticosteroids in the 5α- and 5β-Series

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Here, we describe the chemical synthesis of the complete sets of 18 novel 3- and 21-monosulfates and their double-conjugated form of tetrahydrocortisol (THF), tetrahydro-11-deoxycortisol (THS), and tetrahydrocortisone (THE) in the 5α- and 5β-series. The principal reactions involved are: (1) selective protection of a specific hydroxy group in substrates; (2) catalytic hydrogenation at C-5 of Δ^4-3-ketosteroids with 10% $Pd(OH)_2$/C to yield 3-oxo-5β-steroids and reductive allomerization with 10% Pd/C to yield 3-oxo-5α-isomers; (3) reduction of the resulting 3-oxo-5β- and 3-oxo-5α-steroids to the corresponding 3α-hydroxy-compounds with $Zn(BH_4)_2$ and K-Selectride®, respectively; and (4) sulfation of hydroxy groups at C-3 and/or C-21 in the tetrahydrocorticosteroid derivatives with sulfur trioxide-triethylamine complex.

Key words sulfate; catalytic reduction; sulfur trioxide-triethylamine complex; tetrahydrocortisol; tetrahydrocortisone; tetrahydro-11-deoxycortisol

As the principal glucocorticoid in humans, cortisol plays a crucial role in modulating the metabolic and homeostatic process that protects against stress, shock, inflammation, *etc.*[3—7)] It is synthesized primarily in the zona fasciculata with a small contribution from the zona reticularis.[3)] While cortisol is essentially secreted by the adrenal glands, cortisone is mainly produced using 11β-hydroxysteroid dehydrogenase isoenzymes, which interconvert cortisol to hormonally inactive cortisone.[3)] Cortisol and cortisone are extensively metabolized to tetrahydro-reduced derivatives: tetrahydrocortisol (THF) and tetrahydrocortisone (THE), and their 5α-stereoisomer (allo-THF and allo-THE, respectively).[3,7—9)] Metabolism decreases the biological activity of hormones and increases their water solubility by converting them to hydrophilic compounds that can be excreted in urine. Unmetabolized cortisol and cortisone comprise only *ca.* 0.1% of the total urinary cortisol metabolites. At least 90% of the tetrahydro-derivatives of cortisol and cortisone metabolites are excreted into the urine as sulfate or glucuronide conjugates.[10—13)] Tetrahydro-11-deoxycortisol (THS) and its 5α-stereoisomer (allo-THS) which are tetrahydro-reduced metabolites of 11-deoxycortisol (11-DOC), a biosynthetic precursor of cortisol, are also excreted in urine as conjugated forms.[8,10,12)] In order to obtain information regarding the related biochemistry, physiology, and pathophysiology of endocrine disorders, it is necessary to identify all the corticosteroid metabolites found in urine, and determine the exact structure of their conjugates, their concentration, and the dynamics of their formation and disposal.

At present, analytical methods for the detection of conjugated tetrahydrocorticosteroids are based on gas chromatography and mass spectrometric determination of hydrolyzed and derivatized compounds.[14—18)] Although these methods are robust and sensitive, sample preparation in these indirect methods is time consuming, and the GC-MS sample throughput is relatively low. Thus the development or more straightforward methods based on the direct analysis of steroid conjugates without the need for a deconjugation process is of great interest. The application of liquid chromatographic separation interfaced by soft ionization techniques, such as electrospray ionization (ESI) with tandem mass spectrometry and/or ion trap mass spectrometry, offers an effective analytical tool for the direct monitoring of the urinary conjugates of tetrahydrocorticosteroids.[19,20)] Recently, we reported a highly sensitive and specific liquid chromatography/ESI-linear ion trap mass spectrometry method for the direct measurement of the glucuronide conjugates of THF, THE, THS, and their 5α-stereoisomer in human urine.[21)] The authentic glucuronides had been chemically synthesized by Hosoda *et al.*[22—25)] One significant drawback of sulfate conjugate analysis, however, is still the lack of reference materials, which are essential for the development and application of analytical methods. In addition, conjugated reference standards are also needed within the field of steroid metabolism research. The objective of this study was to synthesize 18 sulfated conjugates of tetrahydrocorticosteroids as shown in Fig. 1. The compounds selected in this study include possible and potential metabolites, some of which have already been detected in human urine.[3,8,10)]

Results and Discussion

For the purpose of a highly sensitive, selective, and accurate determination of the polar and hydrophilic sulfated conjugates of tetrahydrocorticosteroids, the best analytical method seemed to be the use of LC-MS/MS coupled with the available authentic reference standards. As part of our ongoing program to prepare new or scarce corticosteroid conjugates as potential metabolites that have clinical utility, we carried out the chemical synthesis of the 18 variants of the 3- and 21-monosulfate conjugates as well as the double-con-

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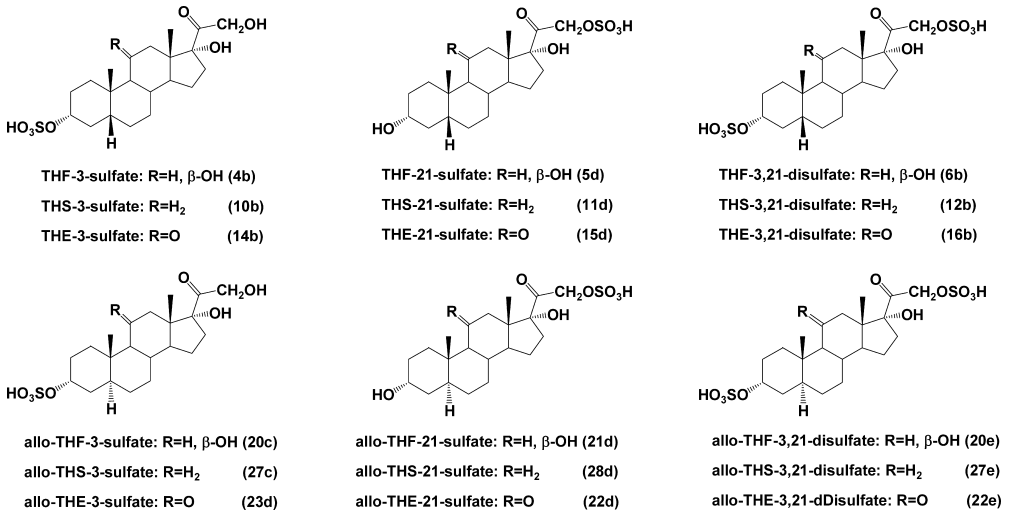


Fig. 1. Chemical Structures of the Desired Sulfates of Tetrahydrocorticosteroids

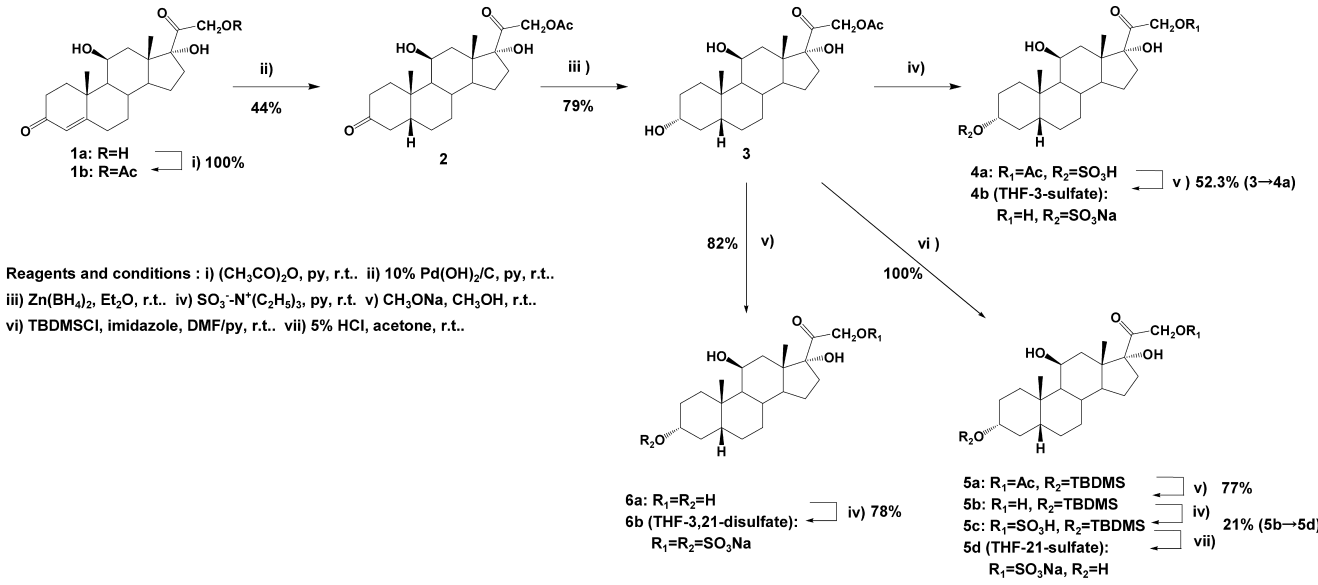


Fig. 2. Synthetic Route to the Sulfate Conjugates of THF

jugated 3,21-disulfates of THF, THS, and THE, and their corresponding "*allo*" series of 5α-stereoisomers (see Fig. 1).

The desired THF-3- and THF-21-sulfates (**4b**, **5d**) and THF-3,21-disulfate (**6b**) were prepared from the 21-acetoxy derivative (**1b**) of cortisol (**1a**), according to the route depicted in Fig. 2. A key intermediate in the synthesis of the sulfated THFs was 21-acetoxy-3α,11β,17α-trihydroxy-20-one (**3**), which was prepared from **1a** in three steps. The transformation of conjugated Δ^4-3-ketosteroids into the stereoisomeric 3-oxo-5β- (normal *cis* A/B-ring juncture) and 3-oxo-5α-steroids (*allo trans* A/B-ring juncture) has been improved by many groups of workers. It is now well recognized that the formation ratio of 3-oxo-5β-/3-oxo-5α-steroids is significantly influenced not only by the structures of the Δ^4-3-ketosteroids but also the reducing agents that are employed. Combe *et al.*[26] have recommended the use of $Pd/CaCO_3$ as a hydrogenation catalyst for the reduction of Δ^4-3-ketosteroids into their corresponding 3-oxo-5β-steroids. Hosoda *et al.*[22,23] have carried out a procedure for the preparation of 21-acetoxy-11β,17α-dihydroxy-5β-pregnane-3,20-dione (**2**) and 21-acetoxy-17α-hydroxy-5β-pregnane-3,20-dione (**8**) from **1b** and the 21-acetoxy derivative (**7b**) of 11-DOC (**7a**). Our exploratory experiments revealed that the catalytic hydrogenation of **1b** and **7b** in the presence of 5% $Pd/CaCO_3$ was unsatisfactory, and the reduction reaction did not proceed at all. However, by changing the catalyst to 10% $Pd(OH)_2/C$, reduction of **1b** and **7b** proceeded smoothly to yield the desired **2** and **8**, respectively. The use of $Pd(OH)_2/C$ was found to be much preferred over that of $Pd/CaCO_3$.

Conversion of 3,20-dioxo-5β-steroid (**2**) to a key intermediate, 3α-hydroxy-20-oxo-5β-steroid (**3**), has previously been achieved by the reduction of **2** using a Raney nickel catalyst.[22] However, the reduction of **2** by zinc borohydride ($Zn(BH_4)_2$)[27,28] offered an easier and more practical procedure for the preparation of **3**. This less basic, mild zinc reagent, compared to common $NaBH_4$, leaves the oxo group at C-20 in **2** intact at room temperature for 45 min during the reduction at C-3. In addition, $Zn(BH_4)_2$ reduction proceeds more simply and efficiently than with catalytic hydrogenation by Raney nickel. The α-configuration of the C-3 hydroxy

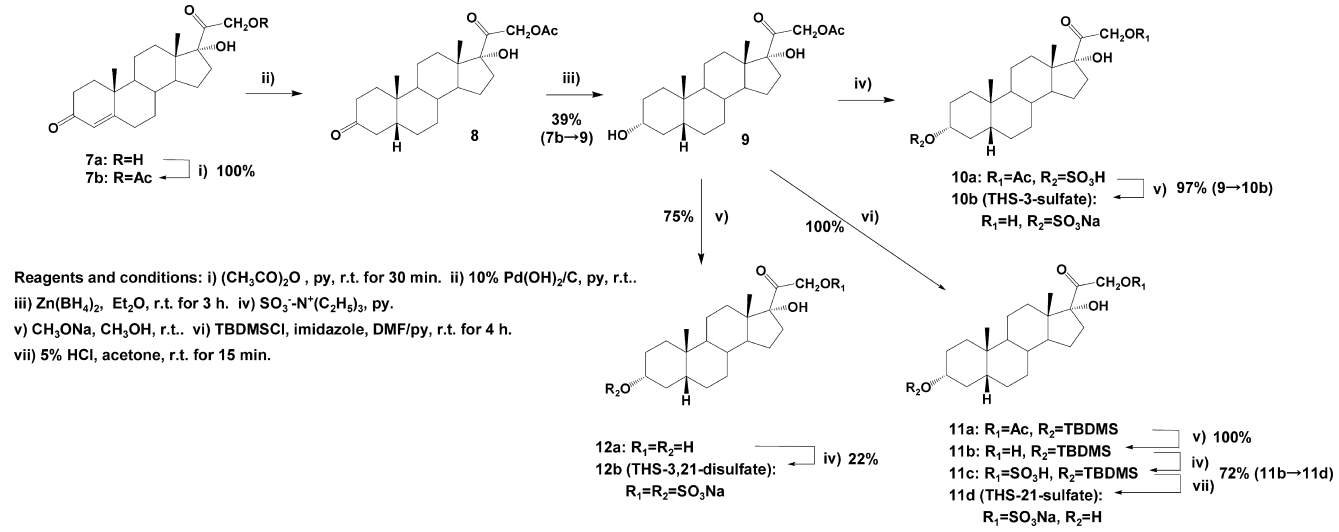


Fig. 3. Synthetic Route to the Sulfate Conjugates of THS

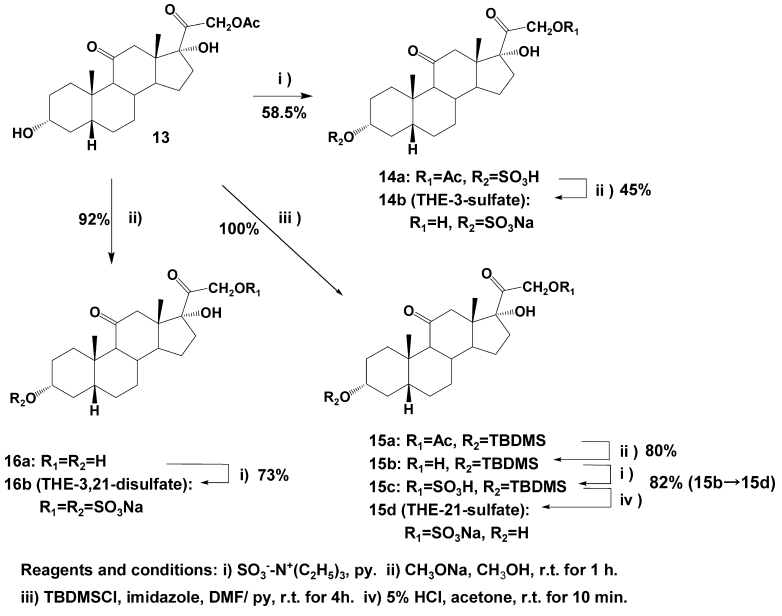


Fig. 4. Synthetic Route to the Sulfate Conjugates of THE

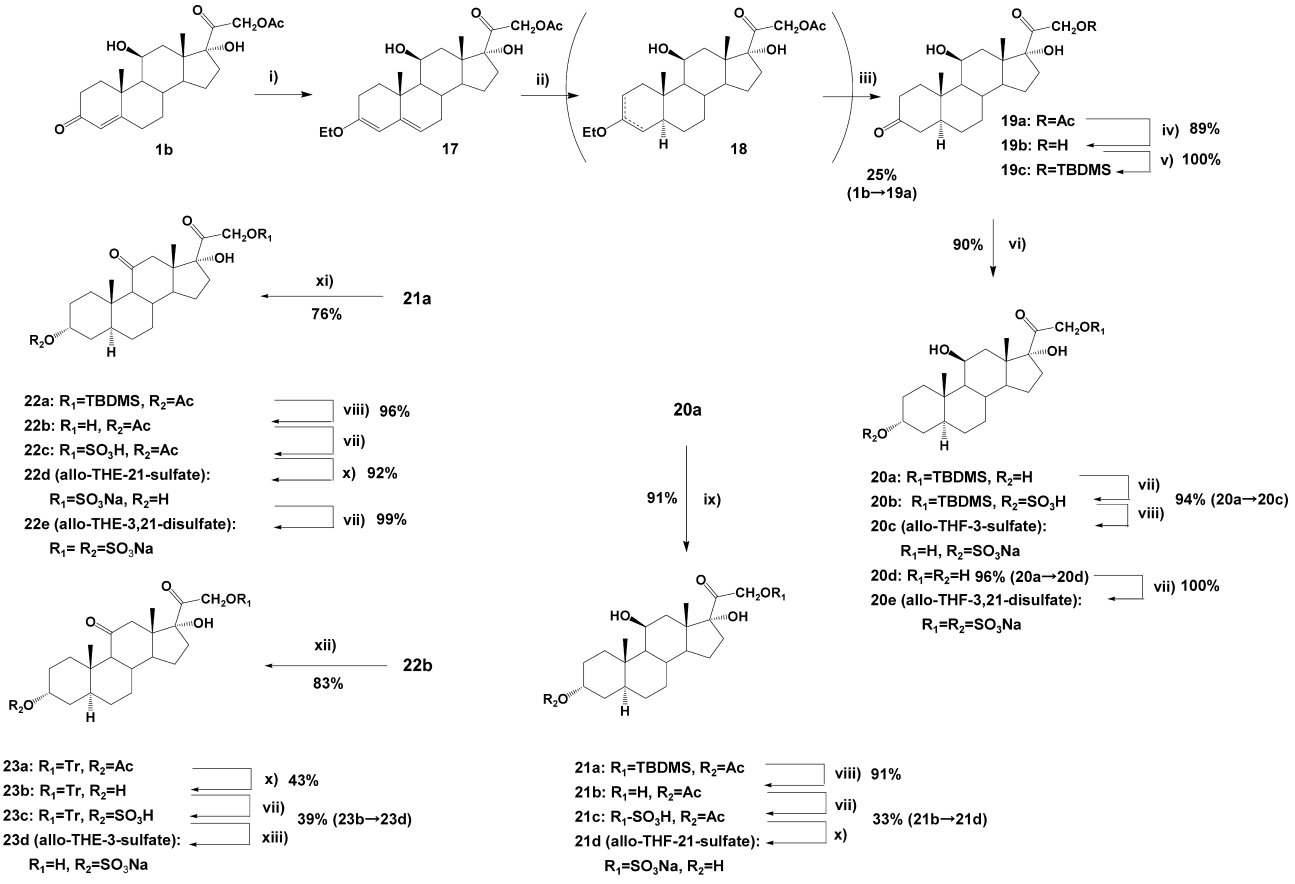
group in **3** was characterized by a 3β-H signal appearing at 3.64—3.73 ppm as a broad multiplet with the half-band width of *ca.* 20 Hz in the ^{1}H-NMR spectrum.

The 3α-hydroxy group in **3** was selectively sulfated with sulfur trioxide-triethylamine (SO_3-TEA) complex in pyridine. The sulfation reaction proceeded cleanly and rapidly under mild experimental conditions. The resulting 3-sulfated compound (**4a**) was then hydrolyzed with sodium methoxide to remove the C-21 acetoxy group. To purify the crude THF-3-sulfate (**4b**), it was loaded onto an Oasis® HLB cartridge for reversed-phase solid phase extraction. After the cartridge was washed with water to remove excess reagents and inorganic salts, elution with methanol yielded a homogeneous effluent, which was characterized as THF-3-sulfate (**4b**).

Meanwhile, compound **3** was selectively silylated at C-3 by *tert*-butyldimethylsilyl chloride (TBDMSCl) to obtain 21-acetoxy-3α-TBDMSO-11β,17α-dihydroxy-5β-20-one (**5a**), which in turn was hydrolyzed with sodium methoxide to afford 3α-TBDMSO-11β,17α,21-trihydroxy-20-one (**5b**). Treatment of **5b** with SO_3-TEA complex yielded 3α-TBDMSO-11β,17α-hydroxy-21-sulfate (**5c**). Subsequent desilylation at C-3 in **5c** with HCl resulted in the formation of THF-21-sulfate (**5d**). Furthermore, alkaline hydrolysis of **3** with sodium methoxide, followed by sulfation of the resulting THF (**6a**) with SO_3-TEA complex, afforded THF-3,21-disulfate (**6b**).

Essentially identical procedures were used to prepare the 3- and 21-monosulfates and 3,21-disulfates of THS (**10b**, **11d**, **12b**) and THE (**14b**, **15d**, **16b**), starting from 11-DOC (**7a**) and THE-21-acetate (**13**), respectively, as outlined in Figs. 3 and 4.

The focus of our next synthetic targets was the "*allo*" series of the sulfate conjugates of tetrahydrocorticosteroids, which are the 5α-stereoisomers of sulfate-conjugated THF, THS, and THE, as shown in Fig. 5. Of various methods for the reductive allomerization at C-5 of Δ^4-3-ketosteroids reported previously,[24,25,29,30] it seemed to us that the most feasible route, starting from the readily available **1b** through



Reagents and conditions: i) Triethyl orthoformate, *p*-TsOH, THF-EtOH, r.t. for 2h. ii) 10% Pd/C, EtOAc-EtOH, r.t.. iii) 7.3% HCl. iv) CH_3ONa, CH_3OH, r.t.. v) TBDMSCl, imidazole, DMF/py, r.t.. vi) K-Selectride , tetrahydrofuran, -78°C. vii) SO_3-$N(C_2H_5)_3$, py, r.t.. viii) 5% HCl, acetone, r.t..x) $(CH_3CO)_2O$, py, r.t.. x) NaOH, CH_3OH, r.t.. xi) PCC, CH_2Cl_2, r.t. for 1.5 h. xii) Tritylchloride, py, 60°C for 3 h. xiii) c.HCl-EtOH, r.t. for 2 h.

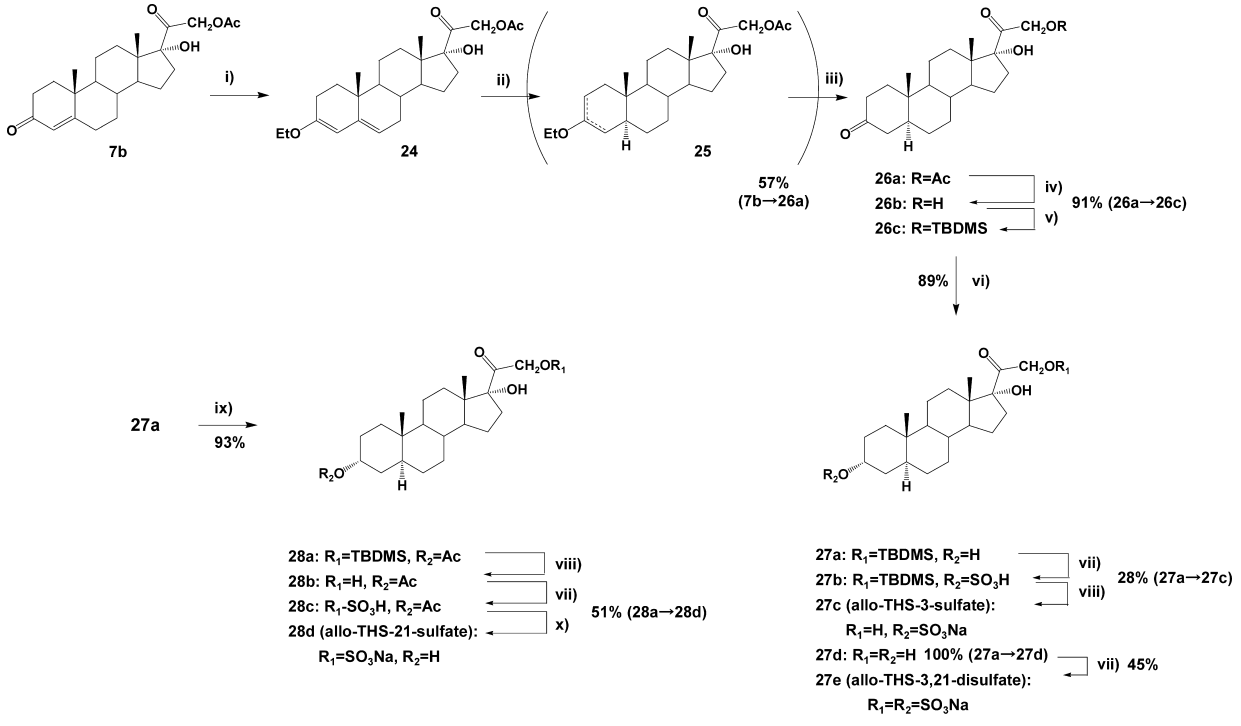
Fig. 5. Synthetic Route to the Sulfate Conjugates of Allo-THF and Allo-THE

the inversion of the configuration at C-5, offered a better way to obtain the 3-ethoxy-3,5-diene intermediate (**17**).[24,25] The procedures employed were (1) the etherification of **1b** with triethyl orthoformate in the presence of a catalytic amount of *p*-toluenesulfonic acid and (2) selective reduction of the $\Delta^{3,5}$-bond in the resulting **17** over 10% Pd/C catalyst, yielding enol ether (**18**), which after treatment with HCl gave the desired **19a**. After chromatographic purification, the allomerized 3-oxo-5α compound (**19a**) was isolated in a fairly good yield.

A sterically bulky hydride reducing agent, potassium tri-*sec*-butylborohydride (K-Selectride®), is known to have high stereoselectivity in the reduction of oxo to the hydroxy group.[24,25,30,31] For instance, 3-oxo-5β- and 3-oxo-5α-steroids are reduced to 3β-hydroxy-5β- and 3α-hydroxy-5α-isomers, respectively.[32] In fact, when 21-TBDMSO-11β,17α-dihydroxy-5α-3,20-dione (**19c**) was subjected to reduction with K-Selectride®, the major isolated product was the axially 3α-hydroxylated product (**20a**). The α-configuration at C-3 in **20a** was confirmed by the equatorial 3β-H signal appearing at 4.03—4.08 ppm as a multiplet with the half-band width of *ca.* 8 Hz in the ^{1}H-NMR spectrum. Prior to the reduction reaction of **19c**, the C-21 acetoxy group in **19a** was hydrolyzed with sodium methoxide, then the C-21 hydroxy group of the resulting 11β,17α,21-trihydroxy-5α-3,20-dione (**19b**) was protected with TBDMSCl to give **19c**. Treatment of **20a** with SO_3-TEA complex, followed by desilylation of the resulting 3α-sulfooxy derivative (**20b**) with HCl, afforded the desired allo-THF-3-sulfate (**20c**).

Allo-THF-21-sulfate (**21d**) was prepared by the following sequence steps: (1) acetylation at C-3 in **20a**, (2) desilylation at C-21 in **21a**, (3) sulfation at C-21 in **21b**, and then (4) alkaline hydrolysis at C-3 in **21c**. Allo-THF-3,21-disulfate (**20e**) was similarly obtained by desilylation at C-21 in **20a** and sulfation at both C-3 and C-21 in **20d**. The preparation of allo-THE-21-sulfate (**22d**) and allo-THF-3,21-disulfate (**22e**) was also attained starting from 3α-acetoxy-21-TBDMSO-17α-hydroxy-5α-11,20-dione (**22a**) by the essentially identical procedures described for the preparation of THF-21-sulfate (**5d**) and THF-3,21-disulfate (**6b**). Selective oxidation of the 3α-acetoxy-21-TBDMSO-11β,17α-dihydroxy-5α-20-one (**21a**) with pyridinium chlorochromate (PCC) under mild experimental conditions provided **22a** in good isolated yield.

Based on the above findings, allo-THE-3-sulfate (**23d**) could be expected to be prepared from **22a** in several steps. Unexpectedly, a preliminary experiment revealed that the attempted alkaline hydrolysis of the C-3 acetoxy group in **22a** yields a complex mixture, probably arising from the decomposition products, from which the expected 21-TBDMSO-



Reagents and conditions: i) Triethyl orthoformate, c. H_2SO_4, dioxane-EtOH, r.t. for 1 h. ii) 10% Pd/C, EtOAc-EtOH, r.t.. iii) 12% HCl. iv) 1% NaOH, CH_3OH, r.t.. v) TBDMSCl, imidazole, DMF/py, r.t.. vi) K-Selectride , tetrahydrofuran, -80°C for 30 min. vii) $SO_3^-N(C_2H_5)_3$, py, r.t.. viii) 10% HCl, acetone, r.t.. ix) $(CH_3CO)_2O$, py, r.t.. x) 10% NaOH, CH_3OH, r.t..

Fig. 6. Synthetic Route to the Sulfate Conjugates of Allo-THS

Table 1. ^{13}C-NMR Chemical Shifts for the 3- and 21-Mono- and 3,21-Disulfate Conjugates[a)]

Carbon	4b	5d	6b	10b	11d	12b	14b	15d	16b	20c	21d	20e	23d	22d	22e	27c	28d	27e
1	34.7	34.6	34.6	35.1	35.0	35.0	35.7	35.8	35.8	33.2	32.8	33.3	32.5	32.0	32.5	33.9	33.5	33.9
2	29.3	31.7	29.4	28.9	31.2	28.9	29.4	31.8	29.4	27.7	29.3	27.7	27.6	29.3	27.6	27.9	29.7	27.9
3	80.4	72.5	80.5	80.3	72.4	80.4	80.0	72.0	80.2	76.5	67.2	76.5	76.1	67.0	76.2	76.3	67.2	76.4
4	34.6	37.2	34.6	34.6	37.2	34.6	34.7	37.3	34.8	34.2	37.4	34.2	34.1	36.9	34.1	34.6	37.2	34.6
5	44.9	44.9	44.9	43.6	43.6	43.7	44.6	44.6	44.7	41.6	41.1	41.6	40.6	40.1	40.6	40.8	40.3	40.8
6	27.5	27.6	27.5	27.7	27.8	27.8	27.7	27.8	27.7	29.0	29.3	29.1	28.9	29.1	28.9	29.5	29.6	29.5
7	28.6	28.6	28.5	28.2	28.3	28.2	28.3	28.4	28.3	33.9	34.0	34.0	34.1	34.1	34.1	33.4	33.5	33.4
8	33.1	33.1	33.1	37.3	37.4	37.4	38.2	38.2	38.2	32.8	32.8	32.8	37.9	37.8	37.8	37.0	37.0	37.0
9	46.0	45.9	46.0	41.5	41.6	41.5	52.6	52.7	52.6	59.3	59.3	59.3	65.3	65.4	65.2	55.4	55.5	55.4
10	36.2	36.0	35.9	35.6	35.7	35.6	35.2	35.3	35.3	36.9	37.0	37.0	36.4	36.2	36.5	36.8	36.7	36.8
11	68.3	68.3	68.3	21.7	21.7	21.7	213.8	213.4	213.8	68.4	68.4	68.4	213.9	213.4	213.4	21.6	21.6	21.6
12	41.0	40.8	40.8	32.1	31.9	32.0	51.6	51.4	51.5	40.7	40.5	40.6	51.5	51.4	51.4	32.0	31.8	31.8
13	49.3	49.3	49.3	48.4	48.4	48.4	52.5	52.6	52.6	49.3	49.3	49.3	52.5	52.5	52.5	48.7	48.7	48.7
14	54.0	54.1	54.0	52.2	52.3	52.3	51.6	51.7	51.6	54.0	54.1	54.1	51.7	51.8	51.8	52.3	52.3	52.3
15	24.8	24.7	24.7	24.7	24.7	24.7	24.2	24.1	24.2	24.7	24.7	24.7	24.1	24.1	24.1	24.7	24.6	24.6
16	36.2	36.3	36.2	36.4	36.5	36.5	35.3	35.3	35.3	34.6	34.5	34.6	35.3	35.3	35.3	35.0	34.9	34.9
17	90.6	90.9	90.9	90.7	91.0	91.0	89.5	89.7	89.8	90.5	90.8	90.9	89.4	89.7	89.7	90.7	90.9	90.9
18	17.9	17.7	17.8	15.5	15.3	15.3	16.2	16.0	16.0	14.6	14.4	14.6	11.6	11.4	11.6	11.8	11.7	11.8
19	17.1	27.2	27.1	23.8	23.9	23.8	23.6	23.7	23.6	17.9	17.8	17.8	16.1	16.0	16.1	15.6	15.4	15.4
20	213.1	207.9	207.7	213.5	208.6	208.4	213.0	207.7	207.8	213.2	208.0	207.8	212.9	208.0	207.4	213.6	208.3	208.5
21	67.8	72.0	72.2	67.9	72.2	72.1	67.9	72.2	72.1	67.7	72.0	72.0	67.9	71.9	71.9	67.9	72.1	72.1

a) In ppm downfield from TMS.

3α,17α-dihydroxy-5α-11,20-dione was isolated in only poor yield after tedious chromatographic separation. However, by changing the starting material to 3α-acetoxy-17α,21-dihydroxy-5α-11,20-dione (**22b**), the reactions proceeded cleanly, and the desired **23d** was obtained in three steps. The reactions involved are tritylation of the primary hydroxy group at C-21 in **22b** with triphenylmethyl chloride (trityl chloride) and subsequent alkaline hydrolysis of the resulting 3α-acetoxy-17α-hydroxy-21-trityl-5α-11,20-dione (**23a**) with NaOH to afford 3α,17α-dihydroxy-21-trityl-5α-11,20-dione (**23b**). Sulfation at C-3 in **23b** with SO_3-TEA complex and desilylation of the resulting 17α-hydroxy-3α-sulfooxy-21-trityl ether (**23c**) with HCl yielded allo-THE-3-sulfate (**23d**).

Figure 6 shows the synthetic route to allo-THS-3-sulfate

(**27c**), allo-THS-21-sulfate (**28d**), and allo-THS-3,21-disulfate (**27e**), starting from 11-DOC-21-acetate (**7b**). Synthetic procedures for these compounds are essentially identical to those for the analogous THF sulfates (**20c**, **21d**, **20e**) mentioned above.

In conclusion, a series of the 18 variants of the 3- and 21-monosulfates and 3,21-disulfates of THF, THS, and THE, as well as their 5α-stereoisomers, are now available. These authentic reference standards would be useful for the highly sensitive, selective, and accurate LC-MS/MS determination of the sulfate conjugates of tetrahydrocorticosteroids present in human urine. A further detailed study on a method for the analytical and clinical application is now progressing in our laboratory, and the result will be reported at a later date.

Experimental

Materials Tetrahydrocortisone 21-acetate (THE-21-acetate: **13**) was obtained from Steraloids Inc. (Newport, RI, U.S.A.). Cortisol and 11-deoxycortisol (11-DOC) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Silica gel plates (Merck; F_{254}) and silica gel 60 (Merck; 70—230 mesh) were used for analytical and column chromatography, respectively. A reversed-phase adsorbent, Cosmosil 140C_{18}-OPN, was available from Nacalai Tesque Inc. (Kyoto, Japan). Oasis® HLB cartridges (adsorbent weight, 1 g) and Sep-Pak® tC_{18} cartridges (adsorbent weight, 5 g) were provided by Waters Co. (Milford, MS, U.S.A.) and successively conditioned by washings with methanol and water prior to use. All other chemicals and solvents were analytical grade and obtained from Nacalai Tesque Inc.

Instruments All melting points (mp) were determined on a micro hot-stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-EX270 (Tokyo, Japan) at 270 MHz or JNM-AL400 instrument at 400 MHz, with $CDCl_3$ or CD_3OD containing 0.1% Me_4Si as the solvent; chemical shifts are expressed as δ ppm relative to Me_4Si. The following abbreviations are used: s=singlet, d=doublet, m=multiplet, br m=broad multiplet. ^{13}C-NMR spectra were obtained on a JEOL JNM-EX 270 instrument at 68.8 MHz. The ^{13}C distortionless enhancement by polarization transfer (DEPT; 135°, 90°, 45°) spectra were also measured to determine the ^{1}H signal multiplicity and to differentiate between CH_3, CH_2, CH, and C based on their proton environments. High-resolution mass spectra (HR-MS) were recorded on Shimadzu LC-MS-IT-TOF with electrospray ionization source under the negative ion mode (ESI^-).

Chemical Synthesis. 21-Acetoxy-11β,17α-dihydroxy-5β-pregnane-3,20-dione (2) A solution of cortisol 21-acetate (**1b**, 1.0 g) (prepared from cortisol by the usual acetic anhydride-pyridine method) in pyridine (14.5 ml) was hydrogenated overnight at room temperature in the presence of 10% $Pd(OH)_2$/C catalyst (102 mg). After filtration of the catalyst on Celite, the filtrate was concentrated to dryness under reduced pressure. Recrystallization of the product from ethanol afforded **2** as colorless needles: yield, 445 mg (44 %); mp 220—223 °C (lit.,[22] mp 210—212 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 1.27 (3H, s, 19-CH_3), 2.19 (3H, s, 21-OCOCH_3), 4.37 (1H, m, 11α-H), 4.88 and 5.06 (each 1H, d, J=17.6 Hz, 21-CH_2).

21-Acetoxy-3α,11β,17α-trihydroxy-5β-pregnan-20-one (3) To a freshly prepared solution of $Zn(BH_4)_2$ in Et_2O (7 ml)[27] was added slowly a solution of **2** (700 mg) in dry tetrahydrofuran (8 ml) under N_2 gas, and the mixture was stirred at room temperature for 45 min. The resulting solution was diluted with EtOAc, washed with 5% HCl and water, dried over anhydrous Na_2SO_4, and evaporated to dryness. Purification of the product by column chromatography on silica gel with hexane–EtOAc (1 : 1, v/v) as an eluent and recrystallization of a homogeneous effluent from hexane–EtOAc gave **3** as colorless prisms: yield, 553 mg (79%); mp 197—200 °C (lit.,[22] mp 196—198 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 2.18 (3H, s, 21-OCOCH_3), 3.62—3.73 (1H, br m, 3β-H), 4.33 (1H, m, 11α-H), 4.81 and 5.10 (each 1H, d, J=20.0 Hz, 21-CH_2).

11β,17α,21-Trihydroxy-3α-sulfooxy-5β-pregnan-20-one Sodium Salt (THF-3-sulfate; 4b) To a solution of **3** (50 mg) in dry pyridine (0.5 ml) was added SO_3-TEA complex (30 mg), and the mixture was stirred at room temperature for 30 min. After evaporation of the solvent under reduced pressure, the residue dissolved in a small amount of water was adjusted to pH 8 with 1 M NaOH and loaded onto an Oasis® HLB cartridge. After being washed with water, elution with methanol gave the intermediary 21-acetoxy-3α-sulfate (**4a**). This was hydrolyzed with sodium methoxide (36 mg) in methanol (1 ml) at room temperature for 1.5 h. After evaporation of the solvent, the residue dissolved in water was loaded onto an Oasis® HLB cartridge and washed with water. Elution with methanol and recrystallization of a homogeneous effluent from methanol–Et_2O gave **4b** as colorless amorphous substances: yield, 30 mg (52.3 %); mp 196 °C (dec.). ^{1}H-NMR (CD_3OD) δ: 0.82 (3H, s, 18-CH_3), 1.17 (3H, s, 19-CH_3), 4.25 (1H, m, 11α-H), 4.24—4.38 (1H, br m, 3β-H), 4.29 and 4.66 (each 1H, d, J=19.0 Hz, 21-CH_2). HR-MS (ESI^-), Calcd for $C_{21}H_{33}O_8S$ $[M-Na+H-H]^-$: 445.1896; Found, m/z: 445.1926.

21-Acetoxy-3α-*tert*-butyldimethylsilyloxy-11β,17α-dihydroxy-5β-pregnan-20-one (5a) To a solution of **3** (90 mg) in pyridine (0.2 ml)–*N*,*N*′-dimethylformamide (DMF, 0.3 ml) was successively added imidazole (45 mg) and TBDMSCl (100 mg), and the mixture was stirred at room temperature for 4 h. The resulting slotuion was diluted with Et_2O, washed with water, dried with anhydrous Na_2SO_4, and evaporated to dryness. Recrystallization of the product from acetone gave **5a** as colorless leaflets: yield, 115 mg (*ca.* 100%); mp 200—204 °C (lit.,[22] mp 197—199 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi$(CH_3)_2$), 0.90 (12H, s, 18-CH_3 and 3-OSi-*tert*-Bu), 1.15 (3H, s, 19-CH_3), 2.17 (3H, s, 21-OCOCH_3), 3.57—3.67 (1H, br m, 3β-H), 4.31 (1H, m, 11α-H), 4.80 and 5.06 (each 1H, d, J=16.0 Hz, 21-CH_2).

3α-*tert*-Butyldimethylsilyloxy-11β,17α,21-trihydroxy-5β-pregnan-20-one (5b) A mixture of **5a** (200 mg) and sodium methoxide (50 mg) in methanol (5 ml) was stirred at room temperature for 1 h. After evaporation of the solvent, the residue was diluted with EtOAc, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. Purification of the crude product by column chromatography on silica gel with toluene–acetone (5 : 1, v/v) as an eluent and recrystallization of a homogeneous effluent from ether–hexane gave **5b** as colorless plates: yield, 142 mg (77%); mp 179—181 °C (lit.,[22] mp 187—189 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.07 (6H, s, 3-OSi$(CH_3)_2$), 0.88 (3H, s, 18-CH_3), 0.90 (9H, s, 3-OSi-*tert*-Bu), 1.15 (3H, s, 19-CH_3), 3.57—3.67 (1H, br m, 3β-H), 4.32 (1H, m, 11α-H), 4.29 and 4.66 (each 1H, d, J=19.4 Hz, 21-CH_2).

3α,11β,17α-Trihydroxy-21-sulfooxy-5β-pregnan-20-one Sodium Salt (THF-21-sulfate; 5d) The compound **5b** (50 mg) was treated with SO_3-TEA complex (40 mg) at room temperature for 70 min, as described for the preparation of **4b**. Evaporation of the solvent under reduced pressure afforded the intermediary 21-sulfate (**5c**). Without isolation of **5c**, it was subjected to the desilylation at C-3 with 5% HCl (0.2 ml) in acetone (2 ml) at room temperature for 3 h. After evaporation of acetone under reduced pressure, the resulting solution was adjusted to pH 8 with 1 M NaOH and loaded onto an Oasis® HLB cartridge. After being washed with water, the crude sulfated product was eluted with methanol. Recrystallization of the product from methanol–Et_2O gave **5d** as colorless amorphous substances: yield, 10 mg (21%); mp 164 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 5 : 1, v/v) δ: 0.82 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 3.57—3.67 (1H, br m, 3β-H), 4.28 (1H, m, 11α-H), 4.83 and 5.16 (each 1H, d, J=20.0 Hz, 21-CH_2). HR-MS (ESI^-), Calcd for $C_{21}H_{33}O_8S$ $[M-Na+H-H]^-$: 445.1896; Found, m/z: 445.1876.

3α,11β,17α,21-Tetrahydroxy-5β-pregnan-20-one (THF; 6a) The compound **3** (150 mg) was hydrolyzed with sodium methoxide (43 mg) as described for the preparation of **5b**. After being processed analogously, the crude hydrolysis product was subjected to column chromatography on silica gel with toluene–acetone (3 : 1, v/v) as an eluent. Recrystallization of a homogeneous effluent from acetone gave **6a** as colorless needles: yield, 110 mg (82%); mp 215—217 °C (lit.,[29] mp 202—204 °C). ^{1}H-NMR ($CDCl_3$–CD_3OD; 10 : 1, v/v) δ: 0.83 (3H, s, 18-CH_3), 1.16 (3H, s, 19-CH_3), 3.55—3.66 (1H, br m, 3β-H), 4.28 (1H, m, 11α-H), 4.27 and 4.64 (each 1H, d, J=20.0 Hz, 21-CH_2).

11β,17α-Dihydroxy-3α,21-disulfooxy-5β-pregnan-20-one Disodium Salt (THF-3,21-disulfate; 6b) The compound **6a** (50 mg) was treated with SO_3-TEA complex (50 mg) at room temperature for 1 h, followed by 1 M NaOH, as described for the preparation of **4b**. After passing through an Oasis® HLB cartridge and being washed with water, the desired sulfate was eluted with methanol. Evaporation of the solvent and recrystallization of the product from methanol afforded **6b** as colorless amorphous substances: yield, 61 mg (78%); mp 139—141 °C. ^{1}H-NMR (CD_3OD) δ: 0.74 (3H, s, 18-CH_3), 1.07 (3H, s, 19-CH_3), 4.16 (1H, m, 11α-H), 4.14—4.27 (1H, br m, 3β-H), 4.74 and 4.95 (each 1H, d, J=18.0 Hz, 21-CH_2). HR-MS (ESI^-), Calcd for $C_{21}H_{32}O_{11}S_2$ $[M-2Na+2H-2H]^{2-}$: 262.0693; Found, m/z: 262.0676.

21-Acetoxy-3α,17α-dihydroxy-5β-pregnan-20-one (9) 11-Deoxycortisol 21-acetate (**7b**, 500 mg) (prepared from 11-DOC (**7a**) by the usual acetic anhydride-pyridine method) in pyridine (22 ml) was catalytically hy-

drogenated in the presence of 10% $Pd(OH)_2$/C (50 mg) at room temperature overnight. After filtration of the catalyst on Celite, the filtrate was concentrated to dryness under reduced pressure, yielding the intermediary 3-oxo-5β derivative (**8**). Without purification of **8**, it was reduced with $Zn(BH_4)_2/Et_2O$ solution (1.25 ml) at room temperature for 3 h and processed as described for the preparation of **3**. Purification of the crude product by column chromatography on silica gel with toluene–acetone (3 : 1, v/v) as an eluent and recrystallization of a homogeneous effluent from EtOAc gave **9** as colorless needles: yield, 196 mg (39%); mp 222—225 °C (lit., mp 229—232 °C[23]; 221—224 °C[33]). ^{1}H-NMR ($CDCl_3$) δ: 0.64 (3H, s, 18-CH_3), 0.90 (3H, s, 19-CH_3), 2.15 (3H, s, 21-$OCOCH_3$), 3.56—3.68 (1H, br m, 3β-H), 4.79 and 5.09 (each 1H, d, J=17.7 Hz, 21-CH_2).

17α,21-Dihydroxy-3α-sulfooxy-5β-pregnan-20-one Sodium Salt (THS-3-sulfate; 10b) The compound **9** (50 mg) was subjected to the sulfation with SO_3-TEA complex (30 mg) at 45 °C for 4 h, as described for the preparation of **4a**. After evaporation of the solvent under reduced pressure, the residue dissolved in water was loaded onto an Oasis® HLB cartridge. After being washed with water, the desired sulfate (**10a**) was eluted with methanol. Subsequent hydrolysis of the condensed methanolic solution (*ca.* 1 ml) was attained with sodium methoxide (10 mg) at room temperature for 1 h. The solvent was evaporated and the residue dissolved in water was loaded onto an Oasis® HLB cartridge. After being washed with water, elution with methanol afforded **10b** which was recrystallized from methanol–Et_2O as colorless amorphous substances: yield, 56 mg (97%); mp 196 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD=10 : 1, v/v) δ: 0.61 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 4.18—4.31 (1H, br m, 3β-H), 4.28 and 4.68 (each 1H, d, J=19.4 Hz, 21-CH_2). HR-MS (ESI^-), Calcd for $C_{21}H_{33}O_7S$ $[M-Na+H-H]^-$: 429.1947; Found, *m*/*z*: 429.1926.

21-Acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-5β-pregnan-20-one (11a) The compound **9** (100 mg) was treated with TBDMSCl (90 mg) overnight at room temperature, as described for the preparation of **5a**. After being processed analogously, the product was recrystallized from ethanol to give **11a** as colorless leaflets: yield, 129 mg (*ca.* 100%); mp 191—193 °C (lit.,[23] mp 188—189 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-$OSi(CH_3)_2$), 0.65 (3H, s, 18-CH_3), 0.90 (12H, s, 19-CH_3 and 3-OSi-*tert*-Bu), 2.17 (3H, s, 21-$OCOCH_3$), 3.54—3.63 (1H, br m, 3β-H), 4.82 and 5.09 (each 1H, d, J=17.6 Hz, 21-CH_2).

3α-*tert*-Butyldimethylsilyloxy-17α,21-dihydroxy-5β-pregnan-20-one (11b) The compound **11a** (50 mg) in methanol (3 ml)–tetrahydrofuran (0.5 ml) was treated with sodium methoxide (8 mg) at room temperature for 30 min, as described for the preparation of **5b**. After being processed analogously, the product was recrystallized from Et_2O–hexane to give **11b** as colorless needles: yield, 45 mg (*ca.* 100%); mp 184—186 °C (lit.,[23] mp 187—189 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-$OSi(CH_3)_2$), 0.63 (3H, s, 18-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 0.90 (3H, s, 19-CH_3), 3.54—3.63 (1H, br m, 3β-H), 4.29 and 4.66 (each 1H, d, J=20.0 Hz, 21-CH_2).

3α,17α-Dihydroxy-21-sulfooxy-5β-pregnan-20-one Sodium Salt (THS-21-sulfate; 11d) The compound **11b** (30 mg) was treated with SO_3-TEA complex (50 mg) at 50 °C for 26 h, as described for the preparation of **5d**. After being processed analogously, the resulting 21-sulfate (**11c**) was subjected to the desilylation at C-3 with 5% HCl (0.1 ml) in acetone (1 ml) at room temperature for 15 min. The resulting solution was adjusted to pH 8 with 2.5 M NaOH and poured onto an Oasis® HLB cartridge. After being washed with water, the product eluted with methanol was subjected to column chromatography on silica gel. Elution with $CHCl_3$–methanol (6 : 1, v/v) yielded **11d** as colorless amorphous substances, 21 mg (72%); mp 180 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 5 : 1, v/v) δ: 0.53 (3H, s, 18-CH_3), 0.84 (3H, s, 19-CH_3), 3.43—3.55 (1H, br m, 3β-H), 4.73 and 5.09 (each 1H, d, J=18.2 Hz, 21-CH_2). HR-MS (ESI^-), Calcd for $C_{21}H_{33}O_7S$ $[M-Na+H-H]^-$: 429.1947; Found, *m*/*z* 429.1928.

3α,17α,21-Trihydroxy-5β-pregnan-20-one (THS; 12a) The compound **9** (60 mg) was hydrolyzed with sodium methoxide (21 mg) at room temperature for 1.5 h, as described for the preparation of **5b**. After being processed analogously, the crude product was subjected to column chromatography on silica gel with toluene–acetone (3 : 1, v/v) as an eluent. Recrystallization of a homogeneous effluent from methanol gave **12a** as colorless needles: yield, 40 mg (75%); mp 197—199 °C (lit.,[34] mp 222—230 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.62 (3H, s, 18-CH_3), 0.92 (3H, s, 19-CH_3), 3.54—3.66 (1H, br m, 3β-H), 4.26 and 4.66 (each 1H, d, J=19.7 Hz, 21-CH_2).

17α-Hydroxy-3α,21-disulfooxy-5β-pregnan-20-one Disodium Salt (THS-3,21-disulfate; 12b) The compound **12a** (21 mg) was treated with SO_3-TEA complex (20 mg) overnight at 40 °C, followed by 1 M NaOH, as described for the preparation of **4b**. After being loaded onto an Oasis® HLB cartridge and washed with water, the desired THS-3,21-disulfate (**12b**) was eluted with methanol. This compound was recrystallized from methanol as colorless amorphous substances: yield, 7.3 mg (22%); mp 184 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 1 : 1, v/v) δ: 0.62 (3H, s, 18-CH_3), 0.93 (3H, s, 19-CH_3), 4.40—4.50 (1H, br m, 3β-H), 4.80 and 5.15 (each 1H, d, J=18.2 Hz, 21-CH_2). HR-MS (ESI^-), Calcd for $C_{21}H_{32}O_{10}S_2$ $[M-2Na+2H-2H]^{2-}$: 254.0719; Found, *m*/*z*: 254.0708.

17α,21-Dihydroxy-3α-sulfooxy-5β-pregnane-11,20-dione Sodium Salt (THE-3-sulfate; 14b) A mixture of THE-21-acetate (**13**, 50 mg) and SO_3-TEA complex (50 mg) in pyridine (1 ml) was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the residue was extracted with EtOAc. The combined extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated to give a first crop of the intermediary 3-sulfate (**14a**). The aqueous layer was passed through Amberlite XAD-2 resin (10 g) and washed with water. Elution with methanol gave a second crop of **14a**: total yield, 35 mg (58.5%). Without further purification, it was hydrolyzed with sodium methoxide (6 mg) in methanol (6 ml) at room temperature for 2 h, as described for the preparation of **5b**. After evaporation of the solvent, the reaction product dissolved in water was passed through a reversed-phase column on Cosmosil 140C_{18}-OPN (10 g) and washed with water. Elution with methanol afforded the crude product, which in turn was chromatographed on silica gel with $CHCl_3$–methanol (3 : 1, v/v) as an eluent. Recrystallization of a homogeneous effluent from methanol–Et_2O gave **14b** as colorless amorphous substances: yield, 26 mg (45%); mp 177 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 5 : 1, v/v) δ: 0.55 (3H, s, 18-CH_3) 1.14 (3H, s, 19-CH_3), 4.21 and 4.65 (each 1H, d, J=20.0 Hz, 21-CH_2), 4.34—4.44 (1H, br m, 3β-H). HR-MS (ESI^-), Calcd for $C_{21}H_{31}O_8S$ $[M-Na+H-H]^-$: 443.1740; Found, *m*/*z*: 443.1730.

21-Acetoxy-3α-*tert*-butyldimethylsilyloxy-17α-hydroxy-5β-pregnane-11,20-dione (15a) THE-21-acetate (**13**, 100 mg) was treated with TBDMSCl (66 mg) at room temperature for 4 h, as described for the preparation of **5a**. After being processed analogously, the product was recrystallized from methanol to give **15a** as colorless leaflets: yield, 128 mg (*ca.* 100%); mp 218—221 °C (lit.,[22] mp 214—216 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-$OSi(CH_3)_2$), 0.60 (3H, s, 18-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.13 (3H, s, 19-CH_3), 2.16 (3H, s, 21-$OCOCH_3$), 3.54—3.63 (1H, br m, 3β-H), 4.62 and 5.13 (each 1H, d, J=17.6 Hz, 21-CH_2).

3α-*tert*-Butyldimethylsilyloxy-17α,21-dihydroxy-5β-pregnane-11,20-dione (15b) The compound **15a** (30 mg) was hydrolyzed with sodium methoxide (5 mg) at room temperature for 50 min, as described for the preparation of **5b**. After being processed analogously, the crude product was chromatographed on silica gel with hexane–EtOAc (2 : 1, v/v) as an eluent. Recrystallization of a homogeneous effluent from acetone gave **15b** as colorless leaflets: yield, 22 mg (80%); mp 209—211 °C (lit.,[22] mp 208—210 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.06 (6H, s, 3-$OSi(CH_3)_2$), 0.58 (3H, s, l8-CH_3), 0.89 (9H, s, 3-OSi-*tert*-Bu), 1.13 (3H, s, 19-CH_3), 3.52—3.63 (1H, br m, 3β-H), 4.24 and 4.64 (each 1H, d, J=20.0 Hz, 21-CH_2).

3α,17α-Dihydroxy-21-sulfooxy-5β-pregnane-11,20-dione Sodium Salt (THE-21-sulfate; 15d) The compound **15b** (25 mg) was treated with SO_3-TEA complex (25 mg) at room temperature overnight, as described for the preparation of **4b**. After evaporation of the solvent under reduced pressure, the resulting 21-sulfate (**15c**) dissolved in acetone (5 ml)–methanol (0.5 ml) was treated with 5% HCl (0.5 ml) at room temperature for 10 min. The mixture was adjusted to pH 8 with 5% $NaHCO_3$, poured onto a column of Cosmosil (5 g), and washed with water. Elution with methanol gave the crude desilylated product, which in turn was chromatographed on silica gel with $CHCl_3$–methanol (2 : 1, v/v) as an eluent. Recrystallization of a homogeneous effluent from methanol–Et_2O gave **15d** as colorless amorphous substances: yield, 20 mg (82%); mp 187 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 5 : 1, v/v) δ: 0.56 (3H, s, 18-CH_3), 1.13 (3H, s, 19-CH_3), 3.55—3.67 (1H, br m, 3β-H), 4.70 and 5.15 (each 1H, d, J=18.5 Hz, 21-CH_2). HR-MS (ESI^-), Calcd for $C_{21}H_{31}O_8S$ $[M-Na+H-H]^-$: 443.1740; Found, *m*/*z*: 443.1729.

3α,17α,21-Trihydroxy-5β-pregnane-11,20-dione (THE; 16a) THE-21-acetate (**13**, 200 mg) was hydrolyzed with sodium methoxide (52 mg) and processed as described for the preparation of **5b**. Recrystallization of the product from acetone–hexane gave **16a** as colorless prisms: yield, 165 mg (92%); mp 190—191 °C (lit.,[35] mp 195—196.5 °C). ^{1}H-NMR δ: 0.55 (3H, s, 18-CH_3), 1.14 (3H, s, 19-CH_3), 3.40—3.52 (1H, br m, 3β-H), 4.19 and 4.62 (each 1H, d, J=19.7 Hz, 21-CH_2).

17α-Hydroxy-3α,21-disulfooxy-5β-pregnane-11,20-dione Disodium Salt (THE-3,21-disulfate; 16b) THE (**16a**, 38 mg) was treated with SO_3-TEA complex (30 mg) at 50 °C overnight, followed by 1 M NaOH, as described for the preparation of **4b**. The reaction mixture was passed through a column of Cosmosil (10 g) and washed with water. Elution with methanol

gave the crude product, which was then chromatographed on silica gel with $CHCl_3$–methanol (2 : 1, v/v) as an eluent to give **16b** as colorless amorphous substances: yield, 43 mg (73%); mp 167 °C (dec.). ^{1}H-NMR δ: 0.58 (3H, s, 18-CH_3), 1.15 (3H, s, 19-CH_3), 3.97—4.09 (1H, br m, 3β-H), 4.93 and 5.32 (each 1H, d, J=18.4 Hz, 21-CH_2). HR-MS (ESI$^-$), Calcd for $C_{21}H_{30}O_{11}S_2$ $[M-2Na+2H-2H]^{2-}$: 261.0615; Found, *m/z*: 261.0604.

21-Acetoxy-11β,17α-dihydroxy-5α-pregnane-3,20-dione (19a) To a stirred solution of cortisol 21-acetate (**1b**, 2.0 g) in tetrahydrofuran (70 ml)–ethanol (5 ml) was added triethyl orthoformate (7 ml) and *p*-toluenesulfonic acid (75 mg), and the mixture was stirred at room temperature for 2 h. After addition of pyridine (2 ml), the mixture was diluted with EtOAc. The organic layer was washed with 5% $NaHCO_3$ and saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. Purification of the crude product by column chromatography on silica gel with hexane–EtOAc (7 : 3, v/v) as an eluent gave the intermediary 3-ethoxy-3,5-diene (**17**). This was dissolved in EtOAc–ethanol (12 ml; 1 : 1, v/v) and the solution was catalytically hydrogenated on 10% Pd/C (30 mg) overnight at room temperature. After addition of EtOAc (50 ml) followed by removal of the catalyst by filtration on Celite, the filtrate was acidified with 7.3% HCl. The organic layer was washed with 5% $NaHCO_3$ and saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. Purification of the product by column chromatography on silica gel with hexane–EtOAc (3 : 2, v/v) as an eluent and recrystallization of a homogeneous effluent from acetone gave **19a** as colorless prisms: yield, 497 mg (25%); mp 195—197 °C (lit.,[29] mp 220—224 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 18-CH_3), 1.26 (3H, s, 19-CH_3), 2.18 (3H, s, 21-$OCOCH_3$), 4.45 (1H, m, 11α-H), 4.83 and 5.05 (each 1H, d, J=17.6 Hz, 21-CH_2).

11β,17α,21-Trihydroxy-5α-pregnane-3,20-dione (19b) The compound **19a** (205 mg) was hydrolyzed with sodium methoxide (35 mg) as described for the preparation of **5b**. Recrystallization of the crude product from acetone gave **19b** as colorless amorphous substances: yield, 164 mg (89%); mp 229—231 °C (lit.,[25] mp 231—235 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, s, 18-CH_3), 1.26 (3H, s, 19-CH_3), 4.27 and 4.63 (each 1H, d, J=19.6 Hz, 2I-CH_2), 4.41 (1H, m, 11α-H).

21-*tert*-Butyldimethylsilyloxy-11β,17α-dihydroxy-5α-pregnane-3,20-dione (19c) The compound **19b** (450 mg) was treated with TBDMSCl (324 mg) at room temperature for 5 h and processed as described for the preparation of **5a**. Recrystallization of the product from acetone gave **19c** as colorless needles: yield, 597 mg (*ca.* 100%); mp 198—200 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.12 (6H, m, 21-$OSi(CH_3)_2$), 0.93 (12H, s, 18-CH_3 and 21-OSi-*tert*-Bu), 1.26 (3H, s, 19-CH_3), 4.42 and 4.55 (each 1H, d, J=18.0 Hz, 21-CH_2), 4.44 (1H, m, 11α-H).

21-*tert*-Butyldimethylsilyloxy-3α,11β,17α-trihydroxy-5α-pregnan-20-one (20a) To a stirred solution of **19c** (497 mg) in tetrahydrofuran (3.5 ml) at −78 °C was added slowly a 1 M solution of K-Selectride® in tetrahydrofuran (1.5 ml), and the mixture was further stirred at −78 °C for 1.2 h. After gradual addition of 30% H_2O_2 (1.5 ml), the resulting solution was extracted with EtOAc. The combined extract was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated. Purification of the product by column chromatography on silica gel with hexane–EtOAc (2 : 1, v/v) as an eluent and recrystallization of a homogeneous effluent from acetone gave **20a** as colorless prisms: yield, 450 mg (90%). mp 173—176 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.12 (6H, m, 21-$OSi(CH_3)_2$), 0.90 (3H, s, 18-CH_3), 0.93 (9H, s, 21-OSi-*tert*-Bu), 1.03 (3H, s, 19-CH_3), 4.03—4.08 (1H, m, 3β-H), 4.41 and 4.54 (each 1H, d, J=17.8 Hz, 21-CH_2), 4.46 (1H, m, 11α-H).

11β,17α,21-Trihydroxy-3α-sulfooxy-5α-pregnan-20-one Sodium Salt (Allo-THF-3-sulfate; 20c) The compound **20a** (60 mg), treated with SO_3-TEA complex (30 mg) at room temperature for 2 h and processed as described for the preparation of **5d**, yielded the crude 3α-sulfate (**20b**). Without isolation of **20b**, it was subjected to the desilylation at C-21 with 5% HCl (0.2 ml) at room temperature for 1.5 h. The resulting solution was adjusted to pH 8 with 2.5 M NaOH and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give a first crop of the reaction product. Meanwhile, the aqueous layer was passed through an Oasis® HLB cartridge, washed with water, and elution with methanol afforded a second crop. After evaporation of the solvent of the combined extract, the residue was subjected to column chromatography on silica gel. Elution with $CHCl_3$–methanol (3 : 1, v/v) afforded a homogeneous fraction. After evaporation of the solvent, the residue was recrystallized from methanol–Et_2O to give **20c** as colorless amorphous substances: yield, 55 mg (94%); mp 143 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 1 : 5, v/v) δ: 0.83 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 4.28 and 4.65 (each 1H, d, J=18.8 Hz, 21-CH_2), 4.38 (1H, m, 11α-H), 4.59—4.64 (1H, m, 3β-H). HR-MS (ESI$^-$), Calcd for $C_{21}H_{33}O_8S$ $[M-Na+H-H]^-$: 445.1896; Found, *m/z*: 445.1882.

3α,11β,17α,21-Tetrahydroxy-5α-pregnan-20-one (Allo-THF; 20d) A mixture of the compound **20a** (45 mg) and 5% HCl (2 ml) in acetone (0.5 ml) was stirred at room temperature for 10 min. The desilylation product was extracted with EtOAc, and the combined extract was washed with water, dried over anhydrous Na_2SO_4. After evaporation of the solvent, the product was recrystallized from methanol to give **20d** as colorless needles: yield, 33 mg (96%); mp 252—258 °C (lit.,[29] mp 248—256 °C). ^{1}H-NMR ($CDCl_3$–CD_3OD; 1 : 10, v/v) δ: 0.83 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.95—4.00 (1H, m, 3β-H), 4.28 and 4.64 (each 1H, d, J=19.6 Hz, 21-CH_2), 4.39 (1H, m, 11α-H).

11β,17α-Dihydroxy-3α,21-disulfooxy-5α-pregnan-20-one Disodium Salt (Allo-THF-3,21-disulfate; 20e) The compound **20d** (24 mg) was treated with SO_3-TEA complex (58 mg) at room temperature for 2 h, followed by 1 M NaOH, as described for the preparation of **4b**. After loading onto an Oasis® HLB cartridge and being washed with water, the crude product was eluted with methanol. Chromatography of the product on a column of silica gel and elution with $CHCl_3$–methanol (2 : 1, v/v) afforded **20e** as colorless amorphous substances which was recrystallized from methanol–Et_2O: yield, 38 mg (*ca.* 100%); mp 142—143 °C. ^{1}H-NMR ($CDCl_3$–CD_3OD; 2 : 3, v/v) δ: 0.84 (3H, s, 18-CH_3), 1.04 (3H, s, 19-CH_3), 4.39 (1H, m, 11α-H), 4.62—4.67 (1H, m, 3β-H), 4.87 and 5.06 (each 1H, d, J=18.0 Hz, 21-CH_2). HR-MS (ESI$^-$), Calcd for $C_{21}H_{32}O_{11}S_2$ $[M-2Na+2H-2H]^{2-}$: 262.0693; Found, *m/z*: 262.0683.

3α-Acetoxy-21-*tert*-butyldimethylsilyloxy-11β,17α-dihydroxy-5α-pregnan-20-one (21a) A solution of **20a** (404 mg) in pyridine–acetic anhydride (3 ml; 2 : 1, v/v) was stirred overnight at room temperature. After extraction of the acetylation product with EtOAc, the combined extract was washed with 5% $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, and evaporated to give **21a** which was recrystallized from Et_2O–hexane as colorless amorphous substances: yield, 418 mg (91%); mp, 163—164 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.12 (6H, m, 21-$OSi(CH_3)_2$), 0.91 (3H, s, 18-CH_3), 0.94 (9H, s, 21-OSi-*tert*-Bu), 1.04 (3H, s, 19-CH_3), 2.06 (3H, s, 3-$OCOCH_3$), 4.42 and 4.54 (each 1H, d, J=17.6 Hz, 21-CH_2), 4.44 (1H, m, 11α-H), 4.98—5.04 (1H, m, 3β-H).

3α-Acetoxy-11β,17α,21-trihydroxy-5α-pregnan-20-one (21b) The compond **21a** (90 mg), treated with 5% HCl (0.5 ml) at room temperature for 10 min and processed as described for the preparation of **20d**, yielded the desilylation product. Recrystallization from EtOAc gave **21b** as colorless needles: yield, 64 mg (91%); mp 227—229 °C (lit.,[25] mp 228—230 °C). ^{1}H-NMR ($CDCl_3$–CD_3OD; 20 : 1, v/v) δ: 0.86 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 2.06 (3H, s, 3-$OCOCH_3$), 4.27 and 4.63 (each 1H, d, J=19.6 Hz, 21-CH_2), 4.42 (1H, m, 11α-H), 4.98—5.04 (1H, m, 3β-H).

3α,11β,17α-Trihydroxy-21-sulfooxy-5α-pregnan-20-one Sodium Salt (Allo-THF-21-sulfate; 21d) The compound **21b** (50 mg) was treated with SO_3-TEA complex (60 mg) at room temperature for 3 h, followed by 1 M NaOH, as described for the preparation of **4b**. After being processed analogously, the resulting 21-sulfate (**21c**) dissolved in methanol (1 ml) was hydrolyzed with 5 M NaOH (0.3 ml) at room temperature for 20 min. The solvent was evaporated under reduced pressure and the residue dissolved in water was applied onto an Oasis® HLB cartridge. After being washed with water, the crude product was eluted with methanol. Chromatography of the product on a column of silica gel and elution with $CHCl_3$–methanol (4 : 1, v/v) afforded **21d** as colorless amorphous substances which was recrystallized from methanol–Et_2O: yield, 19 mg (33%); mp 176 °C. ^{1}H-NMR ($CDCl_3$–CD_3OD; 1 : 10, v/v) δ: 0.84 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 3.94—4.00 (1H, m, 3β-H), 4.39 (1H, m, 11α-H), 4.80 and 5.05 (each 1H, d, J=18.0 Hz, 21-CH_2). HR-MS (ESI$^-$), Calcd for $C_{21}H_{33}O_8S$ $[M-Na+H-H]^-$: 445.1896; Found, *m/z*: 445.1881.

3α-Acetoxy-21-*tert*-butyldimethylsilyloxy-17α-hydroxy-5α-pregnane-11,20-dione (22a) To a solution of **21a** (372 mg) in CH_2Cl_2 (4 ml) was added Celite (500 mg), sodium acetate (19 mg) and PCC (230 mg), and the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with Et_2O and filtered on silica gel. After evaporation of the solvent in the filtrate, the residue was subjected to column chromatography on silica gel with hexane–EtOAc (5 : 1, v/v) as an eluent. Recrystallization of a homogeneous effluent from acetone–hexane gave **22a** as colorless amorphous substances: yield, 283 mg (76%); mp 185—188 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.12 and 0.13 (each 3H, s, 21-$OSi(CH_3)_2$), 0.61 (3H, s, 18-CH_3), 0.93 (9H, s, 21-OSi-*tert*-Bu), 1.01 (3H, s, 19-CH_3), 2.05 (3H, s, 3-$OCOCH_3$), 4.38 and 4.45 (each 1H, d, J=17.6 Hz, 21-CH_2), 4.98—5.03 (1H, m, 3β-H).

3α-Acetoxy-17α,21-dihydroxy-5α-pregnane-11,20-dione (22b) The compound **22a** (60 mg), treated with 5% HCl (0.5 ml) and processed as described for the preparation of **20d**, yielded the crude desilylation product. Recrystallization from acetone–hexane gave **22b** as colorless needles:

yield, 45 mg (96%); mp 187—190 °C (lit.,[25] mp 187—190 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.61 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 2.04 (3H, s, 3-$OCOCH_3$), 4.25 and 4.64 (each 1H, d, J=20.0 Hz, 21-CH_2), 4.98—5.03 (1H, m, 3β-H).

3α,17α-Dihydroxy-21-sulfooxy-5α-pregnane-11,20-dione Sodium Salt (Allo-THE-21-sulfate; 22d) The compound **22b** (35 mg), subjected to the sulfation (at room temperature for 1.5 h) with SO_3-TEA complex (60 mg), followed by 1 M NaOH, and processed as described for the preparation of **4b**, gave the intermediary 3α-acetoxy-21-sulfate (**22c**). Without isolation of **22c**, it was hydrolyzed with 2.5 M NaOH (0.8 ml) in methanol (2 ml) at room temperature for 1 h and then the solution was adjusted to pH 8 with 5% HCl. After being loaded onto an Oasis® HLB cartridge and washed with water, the crude hydrolysis product was eluted with methanol. Chromatography of the product on a column of silica gel and elution with $CHCl_3$–methanol (3 : 1, v/v) afforded the desired allo-THE-21-sulfate (**22d**) which recrystallized from methanol–Et_2O as colorless powders: yield, 47 mg (92%); mp 169 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 2 : 1, v/v) δ: 0.57 (3H, s, 18-CH_3), 0.99 (3H, s, 19-CH_3), 3.95—4.01 (1H, m, 3β-H), 4.70 and 5.12 (each 1H, d, J=18.6 Hz, 21-CH_2). HR-MS (ESI$^-$), Calcd for $C_{21}H_{31}O_7S$ [M−Na+H−H]$^-$: 443.1740; Found, *m/z*: 443.1725.

17α-Hydroxy-3α,21-disulfooxy-5α-pregnane-11,20-dione Disodium Salt (Allo-THE-3,21-disulfate; 22e) Allo-THE-21-sulfate (**22d**, 25 mg), subjected to the sulfation (at room temperature for 20 h) with SO_3-TEA complex (80 mg), followed by 1 M NaOH, and processed as described for the preparation of **4b**, gave the crude product. Chromatography of the product on a column of silica gel and elution with $CHCl_3$–methanol (2 : 1, v/v) afforded allo-THE-3,21-disulfate (**22e**) which recrystallized from acetone–methanol–Et_2O as colorless amorphous substances: yield, 30 mg (99%); mp 145 °C (dec.). ^{1}H-NMR ($CDCl_3$–CD_3OD; 1 : 1, v/v) δ: 0.57 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 4.58—4.63 (1H, m, 3β-H), 4.73 and 5.09 (each 1H, d, J=18.4 Hz, 21-CH_2). HR-MS (ESI$^-$), Calcd for $C_{21}H_{30}O_{11}S_2$ [M−2Na+2H−2H]$^{2-}$: 261.0615; Found, *m/z*: 261.0600.

3α-Acetoxy-17α-hydroxy-21-trityloxy-5α-pregnane-11,20-dione (23a) A mixture of **22b** (113 mg) and trityl chloride (254 mg) in pyridine (0.5 ml) was stirred at 60 °C for 3 h. After extraction of the reaction product with Et_2O, the combined extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The oily residue was subjected to column chromatography on silica gel. Elution with hexane–EtOAc (2 : 1, v/v) gave a homogenous yellow oil which was characterized as the 21-trityl ether (**23a**): yield, 149 mg (83%). ^{1}H-NMR ($CDCl_3$) δ: 0.46 (3H, s, 18-CH_3), 0.98 (3H, s, 19-CH_3), 2.03 (3H, m, 3-$OCOCH_3$), 3.92 and 4.13 (each 1H, d, J=17.6 Hz, 21-CH_2), 4.96—5.01 (1H, m, 3β-H), 7.26—7.46 (15H, m, Ar-H).

3α,17α-Dihydroxy-21-trityloxy-5α-pregnane-11,20-dione (23b) To a solution of **23a** (30 mg) in methanol–tetrahydrofuran (0.6 ml; 1 : 1, v/v) was added 0.75 M NaOH (0.3 ml), and the mixture was stirred at room temperature for 6 h. The resulting solution was neutralized with 5% HCl and extracted with EtOAc. The combined extract was washed with water, dried over anhydrous Na_2SO_4, and evaporated. Chromatography of the residue on a column of silica gel and elution with hexane–EtOAc (1 : 1, v/v) afforded **23b** as colorless amorphous substances: yield, 2.4 mg (43%); mp 145 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.47 (3H, s, 18-CH_3), 0.97 (3H, s, 19-CH_3), 3.92 and 4.13 (each 1H, d, J=17.6 Hz, 21-CH_2), 4.00—4.05 (1H, m, 3β-H), 7.25—7.46 (15H, m, Ar-H).

17α,21-Dihydroxy-3α-sulfooxy-5α-pregnane-11,20-dione Sodium Salt (Allo-THE-3-sulfate; 23d) The compound **23b** (8 mg) was subjected to the sulfation (at room temperature for 4 h) with SO_3-TEA complex (18 mg) as described for the preparation of **4b**. After evaporation of the solvent under reduced pressure, the crude product, 3α-sulfooxy-21-trityl ether **23c**, was treated with ethanol (0.9 ml) containing conc. HCl (0.05 ml) at room temperature for 2 h, and then the solution was adjusted to pH 8 with 0.75 M NaOH. After evaporation of the solvent, the residue was diluted with EtOAc. The aqueous layer was loaded onto an Oasis® HLB cartridge and washed with water. The desired allo-THE-3-sulfate (**23d**) was eluted with methanol and recrystallized from methanol–Et_2O as colorless amorphous substances: yield, 2.4 mg (39%); mp 154 °C. ^{1}H-NMR ($CDCl_3$–CD_3OD; 1 : 1, v/v) δ: 0.56 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 4.22 and 4.60 (each 1H, d, J=19.6 Hz, 21-CH_2), 4.61—4.66 (1H, m, 3β-H). HR-MS (ESI$^-$), Calcd for $C_{21}H_{31}O_8S$ [M−Na+H−H]$^-$: 443.1740; Found, *m/z*: 443.1723.

21-Acetoxy-17α-hydroxy-5α-pregnane-3,20-dione (26a) To a solution of the 11-DOC-21-acetate (**7b**, 5.47 g) in 1,4-dioxane (110 ml) was added triethyl orthoformate (6 ml) and conc. H_2SO_4 (23 μl) in ethanol (4 ml), and the mixture was stirred at room temperature for 1 h. Pyridine (5 ml) was added to the mixture and the reaction product was extracted with EtOAc. The combined extract was washed with 5% $NaHCO_3$ and saturated brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was chromatographed on a column of silica gel. Elution with EtOAc–hexane (1 : 2, v/v) afforded the intermediary 3-ethoxy-3,5-diene (**24**). This compound **24** dissolved in EtOAc (100 ml) and ethanol (100 ml) was catalytically hydrogenated over 10% Pd/C (98 mg) at room temperature for 1 h. After filtration of the catalyst on Celite, the filtrate was acidified with 12% HCl. The precipitate was filtered off to give a first fraction. The mother liquor was extracted with EtOAc, and the combined extract was washed with 5% $NaHCO_3$ and saturated brine, dried over anhydrous Na_2SO_4, and evaporated to give a second fraction. Recrystallization of the combined fraction from EtOAc gave the title compound **26a** as colorless amorphous substances: yield, 3.11 g (57%); mp 246—248 °C (lit.,[24] mp 251—252 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s, 18-CH_3), 1.02 (3H, s, 19-CH_3), 2.17 (2H, s, 21-$OCOCH_3$), 4.83 and 5.09 (each 1H, d, J=17.6 Hz, 21-CH_2).

21-*tert*-Butyldimethylsilyloxy-17α-hydroxy-5α-pregnane-3,20-dione (26c) A solution of the compound **26a** (501 mg) in 1,4-dioxane (60 ml) was hydrolyzed with 1% methanolic NaOH (3 ml) at room temperature for 1 h. The hydrolysis product was extracted with EtOAc, and the combined extract was washed with saturated brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was recrystallized from methanol to give the 17α,21-dihydroxy-3,20-dioxo intermediate (**26b**), which was treated with TBDMSCl (586 mg) at room temperature for 1.5 h and processed as described for the preparation of **5a**, yielded the silylation product. Recrystallization from methanol afforded the title compound **26c** as colorless leaflets: yield, 501 mg (91%); mp 190—192 °C (lit.,[24] mp 191—192 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.12 (6H, s, 21-$OSi(CH_3)_2$), 0.69 (3H, s, 18-CH_3), 0.93 (9H, s, 21-OSi-*tert*-Bu). 1.03 (3H, s, 19-CH_3), 4.42 and 4.55 (each 1H, d, J=18.0 Hz, 21-CH_2).

21-*tert*-Butyldimethylsilyloxy-3α,17α-dihydroxy-5α-pregnan-20-one (27a) The compound **26c** (320 mg) was reduced with 1 M K-Selectride® in tetrahydrofuran (1.3 ml) at −80 °C for 30 min and processed as described for the preparation of **20a**. The crude product was recrystallized from methanol to give **27a** as colorless leaflets: yield, 285 mg (89%); mp 171—172 °C (lit.,[24] mp 175—177 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.11 (6H, s, 21-$OSi(CH_3)_2$), 0.66 (3H, s, 18-CH_3), 0.78 (3H, s, 19-CH_3), 0.93 (9H, s, 21-OSi-*tert*-Bu), 4.02—4.07 (1H, m, 3β-H).

17α,21-Dihydroxy-3α-sulfooxy-5α-pregnan-20-one Sodium Salt (Allo-THS-3-sulfate; 27c) The compound **27a** (182 mg) was treated with SO_3-TEA (253 mg) at room temperature for 3.5 h, as described for the preparation of **4b**. After removal of pyridine by decantation of the mixture diluted with petroleum ether, the precipitated solids (21-TBDMSO-17α-hydroxy-3α-sulfooxy-5α-20-one intermediate, **27b**) were filtered off. To the crude **27b** dissolved in a small amount of methanol was added 10% HCl (0.5 ml), and the mixture was left standing at room temperature for 30 min. The resulting solution was loaded onto a Sep-Pack® tC_{18} cartridge. Elution with methanol–water (3 : 7, v/v) gave a homogenous fraction which was then adjusted to pH 8 with 10% methanolic NaOH. The resulting solution was passed through a Sep-Pak® tC_{18} cartridge and washed with 5% aqueous methanol. Elution with 25% methanol gave **27c** which recrystallized from methanol–EtOAc as colorless amorphous substances: yield, 33 mg (28%); mp 141—144 °C. ^{1}H-NMR (CD_3OD) δ: 0.61 (3H, s, 18-CH_3), 0.82 (3H, s, 19-CH_3), 4.28 and 4.62 (each 1H, d, J=18.9 Hz, 21-CH_2), 4.58—4.62 (1H, m, 3β-H). HR-MS (ESI$^-$), Calcd for $C_{21}H_{33}O_7S$ [M−Na+H−H]$^-$: 429.1947; Found, *m/z*: 429.1932.

3α,17α,21-Trihydroxy-5α-pregnan-20-one (Allo-THS; 27d) The compound **27a** (285 mg), treated with 10% HCl (50 μl) at room temperature for 30 min and processed as described for the preparation of **20d**, yielded the desilylation product. The crude residue was dissolved in water was loaded onto a Sep-Pak® tC_{18} cartridge and elution with 80% methanol afforded **27d** which recrystallized from methanol as colorless amorphous substances: yield, 215 mg (*ca.* 100%); mp 188—189 °C (lit., mp 225—226 °C[24]; mp 203—213 °C[36]). ^{1}H-NMR ($CDCl_3$) δ: 0.66 (3H, s, 18-CH_3), 0.84 (3H, s, 19-CH_3), 4.03—4.07 (1H, m, 3β-H), 4.28 and 4.66 (each 1H, d, J=18.9 Hz, 21-CH_2).

17α-Hydroxy-3α,21-disulfooxy-5α-pregnan-20-one Disodium Salt (Allo-THS-3,21-disulfate; 27e) The compound **27d** (35 mg) was treated with SO_3-TEA (94 mg) at room temperature for 4 h, followed by 1% methanolic NaOH, as described for the preparation of **27c**. After being loaded onto a Sep-pak® tC_{18} cartridge and washed with 5% aqueous methanol, the desired allo-THS-3,21-disulfate (**27e**) was eluted with 20% methanol. This compound was recrystallized from methanol as colorless leaflets: yield, 25 mg (45%); mp 148—151 °C. ^{1}H-NMR (CD_3OD) δ: 0.62 (3H, s, 18-CH_3), 0.83 (3H, s, 19-CH_3), 4.57—4.61 (1H, m, 3β-H), 4.73 and 4.94 (each 1H, d, J=18.9 Hz, 21-CH_2). HR-MS (ESI$^-$), Calcd for

$C_{21}H_{32}O_{10}S_2$ [M−2Na+2H−2H]$^{2-}$: 254.0719; Found, *m/z*: 254.0710.

3α-Acetoxy-21-*tert*-butyldimethylsilyloxy-17α-hydroxy-5α-pregnan-20-one (28a) The compound **27a** (235 mg), treated with acetic anhydride–pyridine at room temperature overnight and processed as described for the preparation of **21a**, yielded the crude acetylation product. Recrystallization from methanol–water gave the title compound **28a** as colorless needles: yield, 239 mg (93%); mp 172—175 °C (lit.,[24] mp 179—180 °C). ^{1}H-NMR ($CDCl_3$) δ: 0.11 (6H, s, 21-OSi(CH_3)$_2$), 0.66 (3H, s, 18-CH_3), 0.79 (3H, s, 19-CH_3), 0.93 (9H, s, 21-OSi-*tert*-Bu), 2.05 (3H, s, 3-OCOCH_3), 4.38 and 4.43 (each 1H, d, J=18.0 Hz, 21-CH_2), 4.92—4.97 (1H, m, 3β-H).

3α,17α-Dihydroxy-21-sulfooxy-5α-pregnan-20-one Sodium Salt (Allo-THS-21-sulfate, 28d) The compound **28a** (53 mg), treated with 10% HCl (8 μl) at room temperature for 1 h and processed as described for the preparation of **20d**, yielded the desilylation product (**28b**). Without purification, it was treated with SO_3-TEA (30 mg) at room temperature overnight, as described for the preparation of **4b**, afforded the intermediary 21-sulfate (**28c**), which was then hydrolyzed with 10% methanolic NaOH (1 ml) overnight at 50 °C. The resulting solution was diluted with water and adjusted to pH 8 with 10% HCl. After being loaded onto a Sep-pak® tC_{18} cartridge and washed with 5% aqueous methanol, the desired allo-THS-21-sulfate (**28d**) was eluted with 40% methanol. This compound was recrystallized from methanol as colorless amorphous substances: yield, 17 mg (51%); mp 201—203 °C. ^{1}H-NMR (CD_3OD) δ: 0.62 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 3.93—3.98 (1H, m, 3β-H), 4.82 and 5.04 (each 1H, d, J=18.9 Hz, 21-CH_2). HR-MS (ESI$^-$), Calcd for $C_{21}H_{33}O_7S$ [M−Na+H−H]$^-$: 429.1947; Found, *m/z*: 429.1933.

The ^{13}C-NMR chemical shifts of the eighteen 3- and 21-monosulfates and their double-conjugates of 3,21-disulfates in the 5α- and 5β-series, assigned on the basis of the parent non-sulfated corticoid analogs,[37] are compiled in Table 1.

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References and Notes

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3) Bondy P. K., "Metabolic Control and Disease," 8th ed., Chap. 2, ed. by Bondy P. K., Rosenberg L. E., W. B. Saunders Co., Philadelphia, PA, 1980, pp. 1427—1499.
4) White P. C., Mune T., Agarwal A. K., *Endocr. Rev.*, **18**, 135—156 (1997).
5) Newell-Price J., Trainer P., Besser M., Grossman A., *Endocr. Rev.*, **19**, 647—672 (1998).
6) Remer T., Maser-Gluth C., Wudy S. A., *Mini Rev. Med. Chem.*, **8**, 153—170 (2008).
7) Stewart P. M., "Williams Textbook of Endocrinology," 11th ed., Chap. 14, ed. by Kronenberg H. M., Melmed S., Polonsky K. S., Larsen P. R., Saunders Elsevia, Philadelphia, PA, 2008, pp. 445—503.
8) Bongiovanni A. M., Cohn R. M., "Chemical and Biological Aspects on Steroid Conjugation," ed. by Bernstein S., Solomon S., Springer-Verlag, New York, 1970, pp. 409—453.
9) White P. C., "Principles and Practice of Endocrinology and Metabolism," 3rd ed., ed. by Becker, K. L., Lippincott Williams & Wilkins, Philadelphia, 2001, pp. 704—714.
10) Kornel L., Miyabo S., Takeda R., *Steroidologia*, **2**, 197—236 (1971).
11) Kornel L., Saito Z., *J. Steroid Biochem.*, **6**, 1267—1284 (1975).
12) Kornel L., Miyabo S., *Steroids*, **25**, 697—706 (1975).
13) Aranoff G., Rösler A., *Acta Endocrinol.*, **94**, 371—375 (1980).
14) Ulick S., Tedde R., Wang J. Z., *J. Clin. Endocrinol. Metab.*, **74**, 593—599 (1992).
15) Shackleton C. H., *J. Steroid Biochem. Mol. Biol.*, **45**, 127—140 (1993).
16) Kasuya Y., Shibasaki H., Furuta T., *Steroids*, **65**, 89—97 (2000).
17) Shibasaki H., Tanabe C., Furuta T., Kasuya Y., *Steroids*, **66**, 795—801 (2001).
18) Rivero-Marabé J. J., Maynar-Mariño J. I., García-de-Tiedra, M. P., Galán-Martín A. M., Caballero-Loscos M. J., Maynar-Mariño M., *J. Chromatogr. B*, **761**, 77—84 (2001).
19) Raffaelli A., Saba A., Vignali E., Marcocci C., Salvadori P., *J. Chromatogr. B*, **830**, 278—285 (2006).
20) Antignac J. P., Le Bizec B., Monteau F., André F., *Steroids*, **67**, 873—882 (2002).
21) Ikegawa S., Hasegawa M., Okihara R., Shimidzu C., Chiba H., Iida T., Mitamura K., *Anal. Chem.*, **81**, 10124—10134 (2009).
22) Hosoda H., Saito K., Ito Y., Yokohama H., Ishii K., Nambara T., *Chem. Pharm. Bull.*, **30**, 2110—2118 (1982).
23) Hosoda H., Yokohama H., Ishii, K., Ito Y., Nambara T., *Chem. Pharm. Bull.*, **31**, 4001—4007 (1983).
24) Hosoda H., Takasaki W., Miura H., Tohkin M., Maruyama Y., Nambara T., *Chem. Pharm. Bull.*, **33**, 4281—4287 (1985).
25) Hosoda H., Osanai K., Fukasawa I., Nambara T., *Chem. Pharm. Bull.*, **38**, 1949—1952 (1990).
26) Combe M. G., Henbest H. R., Jackson W. R., *J. Chem. Soc. C*, **1967**, 2467—2469 (1967).
27) Walker E. R. H., *Chem. Soc. Rev.*, **5**, 23—50 (1976).
28) Iida T., Kakiyama G., Hibiya Y., Miyata S., Inoue T., Ohno K., Goto T., Mano N., Goto J., Nambara T., Hofmann A. F., *Steroids*, **71**, 18—29 (2006).
29) Harnik M., *J. Org. Chem.*, **28**, 3386—3391 (1963).
30) Contreras R., Mendoza L., *Steroids*, **34**, 121—124 (1979).
31) Göndös G., Orr J. C., *J. Chem. Soc., Chem. Commun.*, **1982**, 1239—1240 (1982).
32) Hosoda H., Osanai K., Nambara T., *Chem. Pharm. Bull.*, **39**, 3283—3286 (1991).
33) Harnik, M., *Steroids*, **3**, 359—379 (1964).
34) Bouchard R., Engel Ch. R., *Can. J. Chem.*, **46**, 2201—2206 (1968).
35) Kritchevsky T. H., Garmaise D. L., Gallagher T. F., *J. Am. Chem. Soc.*, **74**, 483—486 (1952).
36) Forchielli E., Rosenkrantz H., Dorfman R. I., *J. Biol. Chem.*, **215**, 713—722 (1955).
37) Blunt J. W., Stothers J. B., *Org. Magn. Reson.*, **9**, 439—464 (1977).