THE INTRODUCTION OF A DOUBLE BOND ON THE STEROID SKELETON – THE PREPARATION OF ENOL SILYL ETHER DERIVATIVES FROM VICINAL DIOLS

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Received December 13, 2010
Accepted March 8, 2011
Published online April 14, 2011

The ways of converting steroid vicinal diol into an unsaturated derivative were studied with the intention of preparing suitable precursors for the introduction of deuterium or tritium into the molecules of brassinosteroids. The model vicinal diol compound, 2α,3α-dihydroxy-5α-pregnane-6,20-dione (**3**), was converted with high regioselectivity to the α-hydroxy ketone derivative, 2α-hydroxy-5α-pregnane-3,6,20-trione (**8**), in a 75% yield. The attempts to convert α-hydroxy ketone **8** to the dioxolene derivative, 2α,3α-(isopropylidenedioxy)-5α-pregn-2-ene-6,20-dione (**6**), failed. The conditions for the conversion of ketone to the corresponding enol trialkylsilyl ether were optimized using cyclohexanone as a model compound. The best and reproducible results were obtained by using *tert*-butyldimethylsilyl trifluoromethansulfonate (TBDMSiOTf) as the silylating reagent and triethylamine as the base. Under these conditions, the 3,6,20-trione (**8**) was converted to 2,3-bis(*tert*-butyldimethylsilyloxy)-5α-pregn-2-ene-6,20-dione (**14**) with a 66% yield.
Keywords: Enol silyl ether; Vicinal diol; Steroids; Brassinosteroids; X-ray diffraction.

Compounds containing one or more double bonds are ideal precursors for preparing tritium-labeled tracers with specific activities higher than 60 Ci/mmol. Tritium is simply introduced by catalytic tritiation with carrier-free tritium gas. Such tracers are indispensable for the mechanism of action studies of highly biologically active compounds with low or medium molecular weight, most often referred to as hormones. The vicinal diol grouping on Ring A is typical for brassinosteroids – plant hormones[1] discovered thirty years ago. In Fig. 1, the formulas of two typical brassinosteroids – 24-epibrassinolide (**1**) and castasterone (**2**) – are given. These compounds have been intensively studied and exhibit not only growth regulation functions in plants but also some promising antiviral activities[2]. However, no synthesis leading to a tritium-labeled brassinosteroid with a

Collect. Czech. Chem. Commun. 2011, Vol. 76, No. 5, pp. 443–456

doi:10.1135/cccc2010145

specific activity higher than 60 Ci/mmol has yet been reported. We decided to exploit the vicinal diol grouping for the introduction of a double bond to the molecule of brassinosteroids. The 2α,3α-dihydroxy-5α-pregnane-6,20-dione (**3**) was chosen as a readily accessible[3] model compound for the elucidation of a transformation pathway leading from the particular brassinosteroid via its enol derivative as a precursor to a tritium-labeled compound.

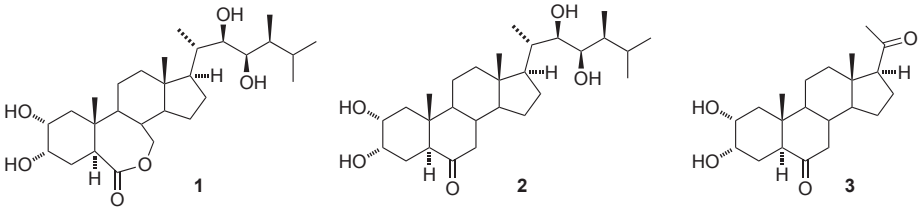
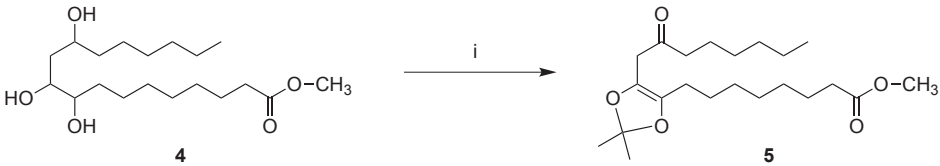


FIG. 1
Formulas of 24-epibrassinolide (**1**), castasterone (**2**) and 2α,3α-dihydroxy-5α-pregnane-6,20-dione (**3**)

The ready formation of the dioxolene ring (Scheme 1) during the oxidation of methyl 9,10,12-trihydroxyoctadecanoate (**4**) by the Jones reagent has been described in the literature[4]. The authors explain the formation of methyl 9,10-(isopropylidenedioxy)-12-oxo-9-octadecenoate (**5**) as the result of the acid-catalyzed reaction of the enol form of the primarily formed α-hydroxy ketone with acetone.



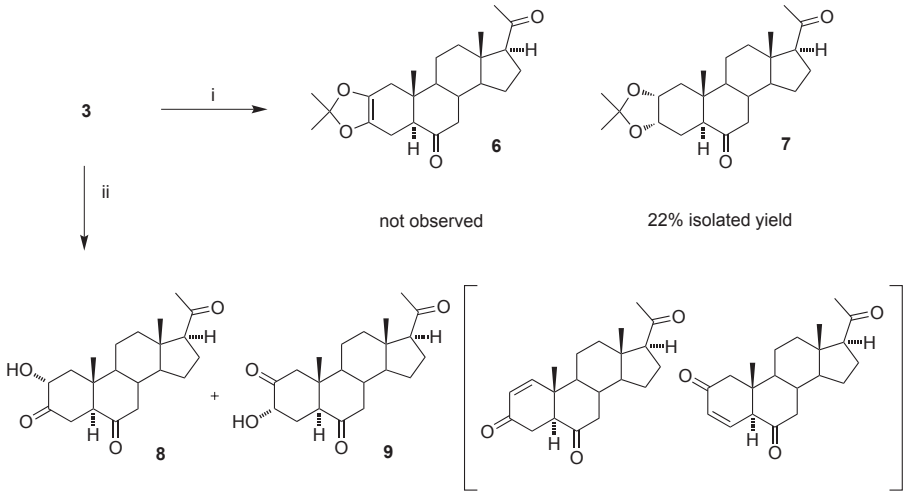
i = CrO_3 , H_2SO_4 , acetone , water, -15 °C

SCHEME 1

RESULTS AND DISCUSSION

The oxidation of dihydroxy pregnane dione **3** by the Jones reagent yielded a complicated mixture of products as revealed by TLC (Scheme 2). Only 2α,3α-(isopropylidenedioxy)-5α-pregnane-6,20-dione (**7**) in a 22% yield and the starting compound **3** in a 44% yield were isolated by chromatogra-

phy on a silica gel, the other compounds decomposed during the workup. We decided to try a two-step conversion of dihydroxy pregnane dione **3** to 2,3-(isopropylidenedioxy)-5α-pregn-2-ene-6,20-dione (**6**). As the first step, we prepared 2α-hydroxy-5α-pregnane-3,6,20-trione (**8**) through the oxidation of dihydroxy pregnane dione **3** by dimethyldioxirane (DMD) (Scheme 2).



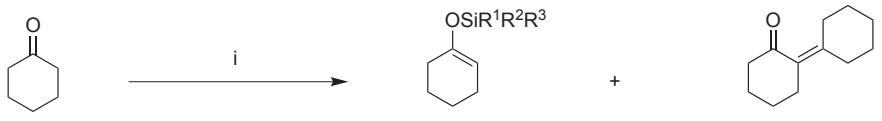
SCHEME 2

When one equivalent of DMD was used for the oxidation, some unreacted starting compound **3** and the regioisomeric 3α-hydroxy-5α-pregnane-2,6,20-trione (**9**) were isolated in addition to 3,6,20-trione **8**. The yield of 3,6,20-trione **8** after chromatographic separation was 54%. The ratio of major 3,6,20-trione **8** to minor 2,6,20-trione **9** was 10:1. The observed high regioselectivity of the oxidation of dihydroxy pregnane dione **3** to 3,6,20-trione **8** is in accordance with the published data on the oxidation of 22,23-(isopropylidenedioxy)-24-epicastasterone with methyl(trifluoromethyl)-dioxirane[5]. When a fourfold excess of DMD was used, the yield of 3,6,20-trione **8** increased to 80% and regioisomer **9** was not observed. Still some unreacted starting compound **3** was present (TLC, about 20% according to NMR). However, no overoxidation to the 2,3-diketo derivative was observed.

Keeping the reaction mixture at 4 °C proved to be important; at r.t., a mixture of products was observed (TLC). At r.t. the rate of disintegration of excess DMD to acetic acid is much more higher and the 3,6,20-trione **8** in acidic solution isomerizes to 2,6,20-trione **9**. It is very likely that both **8** and **9** undergo further acid-catalyzed elimination of the vicinal hydroxy group giving conjugated enones (see the structures in brackets in Scheme 2).

The instability of both α-hydroxy ketones **8** and **9** under acidic conditions is the most probable reason for the unsuccessful attempts to convert 3,6,20-trione **8** to 2,3-(isopropylidenedioxy)-5α-pregn-2-ene-6,20-dione (**6**) by a reaction with acetone under catalysis with 4-toluensulfonic acid (TsOH). We tried to trap the enol form of α-hydroxy ketone **8** also by other methods normally used for the conversion of vicinal diols to the corresponding isopropylidene derivatives[6,7] but without success. These included *trans*-isopropylidenation with 2,2-dimethoxypropane catalyzed with a TsOH, reaction with acetone catalyzed by $FeCl_3$ and a reaction with acetone catalyzed by $Ce(IV)(NH_4)_2(NO_3)_6$. The elimination of condensation water from the reaction mixtures using an azeotropic distillation with benzene or toluene or adding a molecular sieve failed to ameliorate the conditions of the conversion.

We therefore decided to try to convert 3,6,20-trione **8** to another enol derivative – 2,3-bis(trialkylsilyloxy)-5α-pregn-2-ene-6,20-dione – as an alternative possible precursor for catalytic tritiation. Owing to the limited amount of 3,6,20-trione **8** available, we decided to investigate the optimal combination of the trialkylsilyl reagent, base and solvent on cyclohexanone (**10**) as a model compound (Scheme 3).



10

11 $R^1 = R^2 = R^3$ = methyl
12 R^1 = *t*-butyl $R^2 = R^3$ = methyl

13

i = $R^1R^2R^3SiX$, base, solvent, argone atmosphere

TMSiCl	$R^1 = R^2 = R^3$ = methyl		X = Cl
TBDMSiCl	R^1 = *t*-butyl	$R^2 = R^3$ = methyl	X = Cl
TBDMSiOTf	R^1 = *t*-butyl	$R^2 = R^3$ = methyl	X = trifluoromethansulfonyl

SCHEME 3

The results are summarized in Table I. The only reasonable yield was obtained by the use of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSiOTf) as the silylating reagent, triethylamine as the base and dichloromethane (DCM) as the solvent[8]. Thus, these conditions were selected as the starting point for the development of 2,3-bis(*tert*-butyldimethylsilyloxy)-5α-pregn-2-ene-6,2-dione (**14**) synthesis.

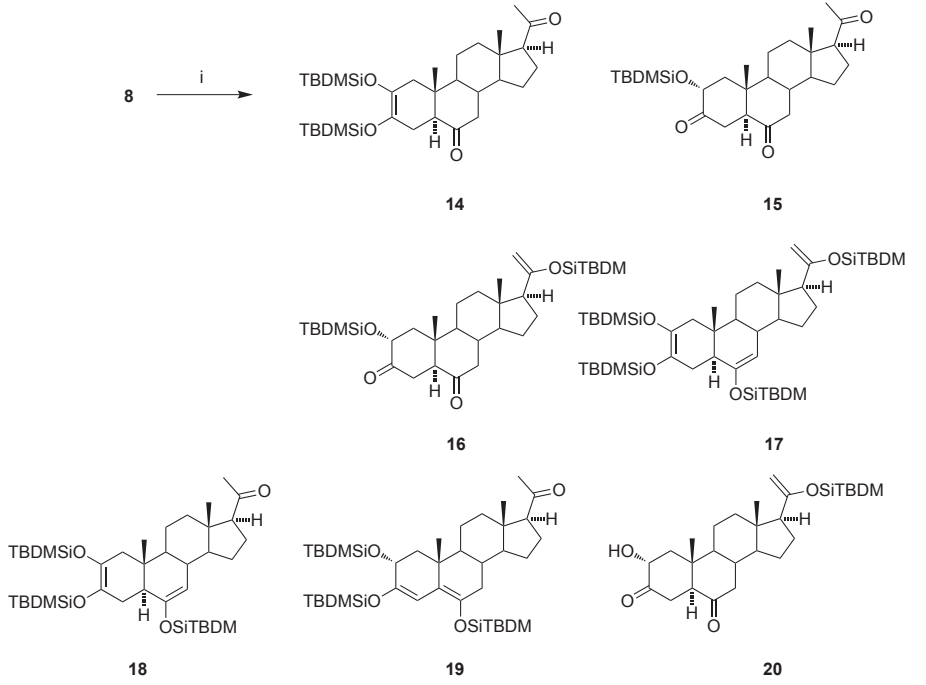
The reaction of 3,6,20-trione **8** with TBDMSiOTf turned out to be strongly dependent on the molar ratio of the reagent to the starting compound and on the mode of mixing all the reaction components together (Scheme 4). In Table II, the results of the pilot experiments are summarized. The conversion of the starting compound to the products was better than 80% in all cases (with one exception). The attempts for the stepwise con-

TABLE I
The results of the reaction of cyclohexanone (**10**) with the silylating reagents under various reaction conditions

Silylation reagent	Reaction conditions	Product yield, %	
		enol **11** or **12**	**13**
TMSiCl (2 eq.)	Et_3N (8 eq.), DCM, r.t., 2 days	<10[a]	0
TMSiCl (2 eq.)	Et_3N (8 eq.), CH_3CN, r.t., 2 days	20[a]	0
TMSiCl (1.2 eq.)	Et_3N (24 eq.), CH_3CN, reflux, 3 days	0[b]	0
TBDMSiCl (3 eq.)	imidazole (6 eq.), CH_3CN, reflux, 3 day	0[b]	0
TBDMSiCl (1.2 eq.)	Et_3N (2.4 eq.), CH_3CN, r.t., 6 days	1[a]	0
TBDMSiCl (1.5 eq.)	imidazole (2 eq.), Et_3N (3 eq.), DCM, sonication, 4 h	<10[b]	0
TBDMSiCl (1.5 eq.)	Et_3N (3 eq.), after 1 h LDA (2 eq.), THF, –78 °C, 3 h	11[c]	50
TBDMSiCl (1.5 eq.)	Et_3N (3 eq.) and LDA (2 eq.), THF, from –78 °C to –5 °C in 3 h	8[c]	0
TBDMSiOTf (1.5 eq.)	Et_3N (1.5 eq.), DCM, r.t., 10 min	83[c]	0

[a] According to ^1H NMR; [b] according to TLC; [c] isolated yield.

version of 3,6,20-trione **8** to the enol **14** via 2α-*tert*-butyldimethylsilyloxy-5α-pregnane-3,6,20-trione (**15**) failed, only 2α,20-bis(*tert*-butyldimethylsilyloxy)-5α-pregn-20-ene-3,6-dione (**16**) was isolated. When larger and larger excesses of TBDMSiOTf to 3,6,20-trione **8** were used, derivatives with enol functions in Rings A and B **17**, **18** and **19** gradually started to appear in the reaction mixtures. The enol **14** was eventually obtained in a satisfactory 66% yield when the molar ratio of TBDMSiOTf to 3,6,20-trione **8** was 40:1, and TBDMSiOTf was added to the reaction mixture in two equal portions. Even though forty equivalents of triethylamine were present, the reaction mixture became slightly acidic at the end. The most plausible explanation of this unexpected result is that 2,3,6,20-tetrakis(*tert*-butyldimethylsilyloxy)-5α-pregna-2,6,20-triene (**17**) (or a mixture of dienes) is formed as a transitional product. When the reaction mixture becomes acidic at the end of the reaction time, the enol groups at C-6 and C-20 are hydrolyzed back to the parent keto groups. The 2,3-bis(*tert*-butyldimethylsilyloxy) enol



i = TBDMSiOTf, base, solvent, argone atmosphere

SCHEME 4

group in **14** is sterically hindered and the double bond is symmetrically substituted with electronegative substituents. This could explain the higher stability of this group in comparison with the *tert*-butyldimethylsilyloxy enol groups on C-20 and C-6.

TABLE II
The results of the reaction of the 3,6,20-trione **8** with TBDMSiOTf

Starting compd.	TBDMSiOTf to steroid ratio	Conditions	Yield, %[a]				
			14	**15**	**16**	mixture of **17**, **18**, **19**	**20**
8	2.2:1	Et_3N (4.4 eq.), 30 min	0	28	37	0	15
15	1.2:1	Et_3N (2.2 eq.), 30 min	0	45	47	0	0
15	3.2:1	Et_3N (4.4 eq.), 30 min	0	18	76	0	0
15	10:1	Et_3N (20 eq.), 30 min	0	4	91	0	0
8	40:1	Et_3N (80 eq.), 1 ml of DCM, 2 h	0	0	0	88[b]	0
8	40:1	Et_3N (80 eq.), 5 ml of DCM, 2 h	0	0	94	0	0
8	40:1	Et_3N (2 × 20 eq.), 1 ml of DCM, TBDMSiOTf 2 × 20 eq. (see Experimental)	66	0	0	0	0

[a] Isolated yield calculated on starting compound; [b] that the mixture of regioisomers had been formed followed from the 1D and 2D NMR.

EXPERIMENTAL

NMR spectra were recorded with a Bruker Avance II 300 MHz spectrometer (^{1}H at 300 MHz, ^{13}C at 75.5 MHz) in $CDCl_3$ unless stated otherwise. Chemical shifts are reported in ppm (δ-scale) relative to TMS. The residual solvent signals in the ^{1}H and ^{13}C NMR spectra were used as an internal reference ($CDCl_3$: δ 7.26 for ^{1}H and δ 77.23 for ^{13}C). The coupling constants (*J*) are given in Hz. The apparent resonance multiplicity is described as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), AB system (A-B quartet) and m (multiplet). Additional NMR techniques such as C-APT, H,H-COSY, H,C-HMBC and H,C-HMQC were used for regular signal assignment. Occasionally, the ^{1}H and ^{13}C NMR spectra showed broad signals or two sets of signals without estimated spin–spin interactions as a result of the hindered rotation of the silyl function. The mass spectra were obtained in the ESI mode either on Q-Tof micro from Waters or on LTQ Orbitrap XL from Thermo Fisher Scientific for the HRMS spectra.

The TLC was performed on Merck Silica gel 60 F_{254} on aluminium sheets or on Merck Aluminium oxide 60 F_{254} neutral on aluminium sheets in a solvent mixture indicated sepa-

rately for each experiment. The detection of the compounds on the TLC plates was first done under a UV_{254} lamp and then the plates were developed by spraying with a 5% solution of phosphomolybdic acid in methanol (w/w) and heating with heat gun set to 350 °C. Flash chromatography was performed on Silica gel 60 from Fluka or on Aluminium oxide Type WN-6, Neutral, Activity Grade Super 1 from Sigma–Aldrich. DCM was dried by $MgSO_4$, distilled and stored over molecular sieve under argon. Tetrahydrofuran (THF) was purified by distillation in a sodium/benzophenone still. Acetonitrile "Gradient grade for HPLC" was purchased from Merck. All of the other reagents were reagent grade purchased from Sigma–Aldrich and used as received. The evaporation of the solvents was done on a rotary evaporator under reduced pressure. The melting points were determined on a Boetius micro-heating block (VEB Analytik, Dresden, Germany) and are uncorrected. The optical rotation values were measured on the AUTOPOL IV (Rudolph Research Analytical, USA) and are given in 10^{-1} deg cm^2/g, the concentrations *c* are given in g/100 ml.

Reaction of 2α,3α-Dihydroxy-5α-pregnane-6,20-dione (**3**) with the Jones Reagent

First, 36 μl of 8 N Jones reagent (prepared from 2.67 g of CrO_3, 2.3 ml of conc. sulfuric acid and diluted with water to the volume of 10 ml) were added to a solution of 100 mg of dihydroxy pregnane dione **3** (provided by Dr. Kohout, prepared according to ref.[3]) in 8 ml of acetone vigorously stirred and cooled to –15 °C under argon atmosphere. The reaction mixture was left for 20 min to warm up to r.t. while being continuously stirred under argon atmosphere. Subsequently, 20 ml of water were added and the reaction mixture was extracted thrice with 5 ml of chloroform. The combined chloroform extracts were washed with 5 ml of a 10% water solution of sodium bicarbonate and dried over $MgSO_4$. TLC (silica gel, chloroform–diethyl ether 1:1) revealed a mixture of products ($0.35 < R_F < 0.7$) besides the residual starting compound **3** (R_F 0.2). After flash chromatography on a silica gel (chloroform–diethyl ether 5:1), the least polar compound – 2α,3α-(isopropylidenedioxy)-5α-pregnane-6,20-dione (**7**) – was isolated (24 mg, 22%), m.p. 238–240 °C, $[\alpha]_D^{20}$ + 93 (*c* 0.32, $CHCl_3$), R_F 0.7 (silica gel; chloroform–diethyl ether 1:1). MS (ESI): 411.2 (100, M + Na), 799.5 (15, 2 × M + Na). HRMS (ESI): for $C_{24}H_{36}O_4Na$ calculated 411.2506, found 411.2506. ^{1}H NMR: 0.61 (3 H, s, CH_3-18); 0.68 (3 H, s, CH_3-19); 1.34 and 1.50 (2 × 3 H, 2 × s, 2 × CH_3 of the isopropylidene); 1.49 and 1.98 (2 × 1 H, 2 × m, CH_2-15); 1.35 and 2.15 (2 × 1 H, 2 × m, CH_2-16); 1.35 and 1.53 (2 × 1 H, 2 × m, CH_2-11); 1.43 (1 H, m, CH-14); 1.45 (1 H, m, CH-9); 1.50 and 2.10 (2 × 1 H, 2 × m, CH_2-12); 1.32 and 2.02 (2 × 1 H, 2 × m, CH_2-1); 2.00 and 2.15 (2 × 1 H, 2 × m, CH_2-4); 1.77 (1 H, m, CH-8); 2.03 and 2.33 (2 × 1 H, m and dd, *J* = 13.2, 4.5, CH_2-7); 2.13 (3 H, CH_3-21); 2.52–2.58 (2 H, m, CH-5α and CH-17α); 4.07–4.14 (1 H, m, CH-2β); 4.27 (1 H, m, CH-3β). ^{13}C NMR: 12.92 (CH_3-19); 13.54 (CH_3-18); 21.33 (CH_2-11); 22.79 (CH_2-16); 22.99 (CH_2-15); 24.39 (CH_2-4); 26.74 and 28.85 (2 × CH_3 of the isopropylidene); 31.65 (CH_3-21); 37.57 (CH-8); 38.66 (CH_2-12); 41.41 (CH_2-1); 42.59 (C-10); 44.46 (C-13); 46.92 (CH_2-7); 51.73 (CH-5); 53.49 (CH-9); 56.98 (CH-14); 63.59 (CH-17); 72.33 (CH-3); 72.49 (CH-2); 108.82 (C of the isopropylidene); 209.15 (CO-20); 211.05 (CO-6).

The starting dihydroxy pregnane dione **3** was isolated back (44 mg, 44%). HRMS (ESI): for $C_{21}H_{32}O_4Na$ calculated 371.2193, found 371.2193. ^{1}H NMR: 0.62 (3 H, s, CH_3-18); 0.76 (3 H, s, CH_3-19); 1.33 and 1.71 (2 × 1 H, 2 × m, CH_2-15); 1.35 and 1.80 (2 × 1 H, 2 × m, CH_2-16); 1.35 and 1.7 (2 × 1 H, 2 × m, CH_2-11); 1.39 (1 H, m, CH-14); 1.40 (1 H, m, CH-9); 1.49 and 2.05 (2 × 1 H, 2 × m, CH_2-12); 1.55 and 1.73 (2 × 1 H, 2 × m, CH_2-1); 1.70 and

1.89 (2 × 1 H, 2 × m, CH_2-4); 1.75 (1 H, m, CH-8); 2.00 and 2.32 (2 × 1 H, m and dd, J = 13.2, 4.5, CH_2-7); 2.14 (3 H, CH_3-21); 2.60 (1 H, t, J = 9.0, CH-17α); 2.70 (1 H, dd, J = 12.6, 3.0, CH-5α); 3.76 (1 H, m, CH-2β); 4.05 (1 H, m, CH-3β). ^{13}C NMR: 13.55 (CH_3-18); 13.73 (CH_3-19); 21.29 (CH_2-11); 22.87 (CH_2-16); 24.26 (CH_2-15); 26.43 (CH_2-4); 31.65 (CH_3-21); 37.62 (CH-8); 38.54 (CH_2-12); 40.24 (CH_2-1); 42.60 (C-10); 44.51 (C-13); 46.67 (CH_2-7); 50.88 (CH-5); 53.66 (CH-9); 56.82 (CH-14); 63.45 (CH-17); 68.30 (CH-2); 68.44 (CH-3); 209.65 (CO-20); 212.02 (CO-6).

2α-Hydroxy-5α-pregnane-3,6,20-trione (**8**)

First, 15 ml of 0.08 M solution of dimethyldioxirane (1.2 mmol, prepared according to ref.[9]) were added to a solution of dihydroxy pregnane dione **3** (415 mg, 1.2 mmol) in 5 ml of DCM, and the reaction mixture was kept in the refrigerator at 4 °C overnight. It was left for 1 h to warm up to r.t. and the solvents were evaporated. Water (20 ml) was added to the oily residue and the resulting emulsion was extracted with DCM (3 × 20 ml). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated. The crude product was purified by flash chromatography on a silica gel (chloroform–hexane–methanol 20:20:1) to afford a pure 3,6,20-trione **8** (240 mg, 58%) as a white solid, m.p. 175–176 °C (chloroform–hexane), $[\alpha]_D^{20}$ + 73 (c 0.31, $CHCl_3$), R_F 0.28 (silica gel; chloroform–hexane–methanol 20:20:1). MS (ESI): 369.2 (100, M + Na), 715.5 (20, 2 × M + Na). HRMS (ESI): for $C_{21}H_{30}O_4Na$ calculated 369.2036, found 369.2035. ^{1}H NMR: 0.63 (3 H, s, CH_3-18); 1.03 (3 H, s, CH_3-19); 1.22 and 1.70 (2 × 1 H, 2 × m, CH_2-15); 1.33 and 1.81 (2 × 1 H, 2 × m, CH_2-16); 1.42 and 1.76 (2 × 1 H, 2 × m, CH_2-11); 1.40 (1 H, m, CH-14); 1.41 (1 H, m, CH-9); 1.45 and 2.03 (2 × 1 H, 2 × m, CH_2-1); 1.49 and 2.10 (2 × 1 H, 2 × m, CH_2-12); 1.73 (1 H, m, CH-8); 2.01 and 2.39 (2 × 1 H, 2 × m, CH_2-7); 2.10 (3 H, s, CH_3-21); 2.53 (1 H, CH-17α); 2.60 (1 H, m, CH-5α); 2.48 and 2.66 (2 × 1 H, 2 × m, CH_2-4); 3.51 (1 H, J = 3.0, OH); 4.22 (1 H, m, CH-2β). ^{13}C NMR: 13.55 (CH_3-18); 14.01 (CH_3-19); 21.93 (CH_2-11); 22.94 (CH_2-16); 24.31 (CH_2-15); 31.57 (CH_3-21); 35.22 (CH_2-4); 37.47 (CH-8); 38.49 (CH_2-12); 42.73 (CH_2-10); 44.48 (C-13); 46.34 (C-7); 47.92 (CH_2-1); 53.47 (CH-9); 56.67 (CH-14); 58.73 (CH-5); 63.40 (CH-17); 72.11 (CH-2); 207.57 (CO-6); 208.88 (CO-20); 210.97 (CO-3).

Regioisomer **9** (24 mg, 6%) was isolated as an oil, $[\alpha]_D^{20}$ + 19 (c 0.07, $CHCl_3$), R_F 0.15 (silica gel; chloroform–hexane–methanol 20:20:1). MS (ESI): 369.2 (100, M + Na), 715.5 (20, 2 × M + Na). HRMS (ESI): for $C_{21}H_{30}O_4Na$ calculated 369.2036, found 369.2035. ^{1}H NMR: 0.64 (3 H, s, CH_3-18); 0.73 (3 H, s, CH_3-19); 1.21 and 1.69 (2 × 1 H, 2 × m, CH_2-15); 1.35 and 1.82 (2 × 1 H, 2 × m, CH_2-16); 1.42 and 1.76 (2 × 1 H, 2 × m, CH_2-11); 1.26 (1 H, m, CH-14); 1.39 (1 H, m, CH-9); 2.36 and 2.10 (2 × 1 H, 2 × m, CH_2-1); 1.48 and 2.13 (2 × 1 H, 2 × m, CH_2-12); 1.73 (1 H, m, CH-8); 2.05 and 2.43 (2 × 1 H, 2 × m, CH_2-7); 2.14 (3 H, s, CH_3-21); 2.56 (1 H, CH-17α); 1.76 and 2.48 (2 × 1 H, 2 × m, CH_2-4); 2.79 (1 H, dd, J = 12.6, 3.0, CH-5α); 3.55 (1 H, J = 3.6, OH); 4.15 (1 H, m, CH-3β). ^{13}C NMR: 13.52 (CH_3-18); 14.49 (CH_3-19); 21.62 (CH_2-11); 22.97 (CH_2-16); 24.31 (CH_2-15); 31.23 (CH_2-4); 31.64 (CH_3-21); 37.62 (CH-8); 38.38 (CH_2-12); 44.33 (C-13); 46.46 (CH_2-10); 46.55 (C-7); 50.75 (CH_2-1); 53.38 (CH-9); 55.47 (CH-5); 56.76 (CH-14); 63.40 (CH-17); 74.69 (CH-3); 208.00 (CO-6); 209.03 (CO-20); 209.77 (CO-2).

Trialkylsilyloxycyclohexene **11** or **12**

The silylating reagent was added to a stirred solution of cyclohexanone (**10**; 100 mg, 1 mmol) in 5 ml of solvent and base under argon atmosphere by a syringe via a rubber septum in

one portion. The ratios of the reagents, temperatures and reaction times are indicated in Table I. The reaction was quenched by adding 10 ml of a 10% water solution of sodium bicarbonate. The mixture was extracted thrice with 15 ml of chloroform, combined chloroform extracts were dried by $MgSO_4$, the solvent was evaporated and the oily residue was applied on a flash silica gel column pretreated with ammonia (the silica gel was mixed with the mobile phase containing 1% of conc. water solution of ammonia and left to stand for 10 min prior to pouring onto the column). After elution with a 10:1 hexane–ethyl acetate mixture, the fractions containing enolate **11** or **12** were evaporated and the oily residue was analyzed by NMR.

tert-Butyldimethylsilyloxy cyclohexene (**12**). ^{1}H NMR: 0.13 (6 H, s, 2 × Me); 0.92 (9 H, s, *t*-Bu); 1.49–1.53 (2 H, m, CH_2); 1.64–1.68 (2 H, m, CH_2); 2.00–2.01 (4 H, m, 2 × CH_2); 4.86–4.89 (1 H, m, CH). It is in agreement with the published data[10,11] (the shift δ 0.01 given for nine hydrogens of *tert*-butyl group in ref.[10] is most likely a typographic error).

Reaction of 2α-Hydroxy-5α-pregnane-3,6,20-trione (**8**) with TBDMSiOTf. General Conditions

The silylating reagent was added to a solution of 3,6,20-trione **8** (20 mg, 58 μmol) and triethyl amine in 2 ml of DCM stirred under inert argon atmosphere by a syringe through a rubber septum. The progress of the reaction was monitored by TLC on aluminium oxide plates. After the reaction completion as monitored by TLC, 10 ml of DCM were added and the reaction mixture was poured into 5 ml of a 10% water solution of sodium bicarbonate.

The organic layer was separated and the water phase was washed with two 5 ml portions of DCM. The combined DCM extracts were dried over $MgSO_4$, the solvent was evaporated and the oily mixture was separated on a chromatography column filled with silica gel or aluminium oxide. The following compounds were separated as chromatographically pure.

2α-tert-Butyldimethylsilyloxy-5α-pregnane-3,6,20-trione (**15**): white solid, m.p. 174–176 °C, $[\alpha]_D^{20}$ + 49 (*c* 0.21, $CHCl_3$), R_F 0.43 (silica gel; hexane–ethyl acetate–methanol 30:10:1). MS (ESI): 483.3 (100, M + Na), 484.3 (30, M + H + Na), 943.6 (10, 2 × M + Na). HRMS (ESI): for $C_{27}H_{44}O_4SiNa$ calculated 483.2901, found 483.2901. ^{1}H NMR: 0.05 and 0.16 (2 × 3 H, 2 × s, $(CH_3)_2Si$); 0.63 (3 H, s, CH_3-18); 0.93 (9 H, s, $(CH_3)_3CSi$); 1.05 (3 H, s, CH_3-19); 1.25 and 1.65 (2 × 1 H, 2 × m, CH_2-15); 1.32 and 1.68 (2 × 1 H, 2 × m, CH_2-16); 1.45 and 1.77 (2 × 1 H, 2 × m, CH_2-11); 1.42 (1 H, m, CH-14); 1.43 (1 H, m, CH-9); 1.67 and 2.26 (2 × 1 H, 2 × m, CH_2-1); 1.47 and 2.10 (2 × 1 H, 2 × m, CH_2-12); 1.86 (1 H, m, CH-8); 2.03 and 2.36 (2 × 1 H, 2 × m, CH_2-7); 2.16 (3 H, s, CH_3-21); 2.40 and 2.63 (2 × 1 H, 2 × m, CH_2-4); 2.54 (1 H, CH-17α); 2.65 (1 H, m, CH-5α); 4.27 (1 H, dd, *J* = 12.0, 6.6, CH-2β). ^{13}C NMR: –5.22 and –4.33 ($(CH_3)_2Si$); 13.60 (CH_3-18); 14.18 (CH_3-19); 18.72 (SiC); 22.03 (CH_2-11); 23.05 (CH_2-16); 24.37 (CH_2-15); 26.02 ($(CH_3)_3C$); 31.62 (CH_3-21); 36.26 (CH_2-4); 37.68 (CH-8); 38.56 (CH_2-12); 42.78 (CH_2-10); 44.53 (C-13); 46.44 (C-7); 49.01 (CH_2-1); 53.69 (CH-9); 56.72 (CH-14); 58.37 (CH-5); 63.47 (CH-17); 73.83 (CH-2); 208.07 (CO-6); 208.55 (CO-20); 209.05 (CO-3).

2α,20-Bis(tert-butyldimethylsilyloxy)-5α-pregn-20-ene-3,6-dione (**16**): white solid, m.p. 127–130 °C, $[\alpha]_D^{20}$ + 13 (*c* 0.25, $CHCl_3$), R_F 0.52 (silica gel; chloroform–hexane–methanol 20:20:1). MS (ESI): 597.4 (100, M + Na), 1171.9 (50, 2 × M + Na). HRMS (ESI): for $C_{33}H_{58}O_4Si_2Na$ calculated 597.3766, found 597.3766. ^{1}H NMR: 0.04 and 0.14 (2 × 3 H, 2 × s, $(CH_3)_2SiOC$-2); 0.18 (6 H, s, $(CH_3)_2SiOC$-20); 0.66 (3 H, s, CH_3-18); 0.91 and 0.94 (2 × 9 H, 2 × s, $(CH_3)_3CSi$); 1.03 (3 H, s, CH_3-19); 1.23 and 1.73 (2 × 1 H, 2 × m, CH_2-15); 1.45 and

1.75 (2 × 1 H, 2 × m, CH_2-11); 1.25 (1 H, m, CH-14); 1.35 (1 H, m, CH-9); 1.80 and 2.02 (2 × 1 H, 2 × m, CH_2-16); 1.63 and 2.39 (2 × 1 H, 2 × m, CH_2-1); 1.47 and 2.05 (2 × 1 H, 2 × m, CH_2-12); 1.88 (1 H, m, CH-8); 2.03 and 2.38 (2 × 1 H, 2 × m, CH_2-7); 2.45 and 2.60 (2 × 1 H, 2 × m, CH_2-4); 2.58 (1 H, m, CH-5α); 2.62 (1 H, CH-17α); 4.04 and 4.11 (2 × 1 H, br s and d, J = 1.2, CH_2-21); 4.28 (1 H, dd, J = 12.0, 6.6, CH-2β). ^{13}C NMR: –5.18 and –4.31 (2 × $(CH_3)_2$Si); 13.37 (CH_3-18); 14.21 (CH_3-19); 18.40 and 18.74 (2 × SiC); 22.09 (CH_2-11); 24.32 (CH_2-16); 24.61 (CH_2-15); 26.04 (($CH_3)_3$C); 36.35 (CH_2-4); 38.15 (CH-8); 38.41 (CH_2-12); 42.95 (CH_2-10); 43.93 (C-13); 46.67 (C-7); 49.08 (CH_2-1); 53.03 (CH-9); 56.03 (CH-14); 56.75 (CH-5); 58.46 (CH-17); 73.92 (CH-2); 90.22 (CH_2-21); 159.43 (C-20); 208.65 (CO-6); 208.72 (CO-3).

2,3,6-Tris(tert-butyldimethylsilyloxy)-5α-pregna-2,6-dien-20-one (**18**): oil, R_F 0.23 (aluminium oxide; hexane–ethyl acetate 50:1). HRMS (ESI): for $C_{39}H_{72}O_4Si_3Na$ calculated 711.4631, found 711.4630. ^{1}H NMR: 0.12 (3 H, s, $(CH_3)_2$SiOC-6); 0.19 and 0.20 (2 × 3 H, 2 × s, $(CH_3)_2$SiOC-2(3)); 0.66 (3 H, s, CH_3-18); 0.81 (3 H, s, CH_3-19); 0.94 and 0.94 (9 H and 18 H, 2 × s, $(CH_3)_3$CSi); 1.90 and 2.30 (2 × 1 H, 2 × m, CH_2-4); 2.12 (3 H, s, CH_3-21); 2.35 (1 H, dd, J = 15.5, 3.9, CH-5α); 2.55 (1 H, t, J = 8.7, CH-17α); 5.76 (1 H, m, CH_2-7). ^{13}C NMR: –4.14, –3.53, –3.40 and –2.98 (3 × $(CH_3)_2$Si); 12.52 (CH_3-18); 13.78 (CH_3-19); 18.53, 18.59 and 18.65 (3 × SiC); 21.37 (CH_2-11); 23.08 (CH_2-16); 24.38 (CH_2-15); 26.09, 26.25 and 26.27 (($CH_3)_3$C); 31.82 (CH_2-21); 35.60 (CH_2-4); 35.76 (CH-8); 39.31 (CH_2-12); 42.94 (CH_2-10); 44.27 (C-13); 45.01 (CH_2-1); 48.05 (CH-9); 51.73 (CH-5); 55.81 (CH-14); 63.87 (CH-17); 104.54 (C-7); 131.14 and 131.76 (C-2 and C-3); 151.37 (C-6); 209.57 (C-20).

2,3,6-Tris(tert-butyldimethylsilyloxy)-5α-pregna-3,5-dien-20-one (**19**): oil, R_F 0.17 (aluminium oxide; hexane–ethyl acetate 50:1). HRMS (ESI): for $C_{39}H_{72}O_4Si_3Na$ calculated 711.4631, found 711.4630. ^{1}H NMR: 0.10, 0.13 and 0.14 (3 × 9 H, 3 × s, $(CH_3)_2$SiOC); 0.64 (3 H, s, CH_3-18); 0.90, 0.91 and 0.96 (3 × 9 H, 3 × s, $(CH_3)_3$CSi); 0.96 (3 H, s, CH_3-19); 1.20 and 2.08 (2 × 1 H, 2 × m, CH_2-1); 2.13 (3 H, s, CH_3-21); 2.55 (1 H, t, J = 8.7, CH-17α); 4.24 (1 H, dd, J = 9.6, 6.3, CH-2); 5.76 (1 H, m, C-4). ^{13}C NMR: –4.07, –3.68, –3.58 and –3.13 (3 × $(CH_3)_2$Si); 13.53 (CH_3-18); 18.53, 18.60 and 18.70 (3 × SiC); 20.19 (CH_3-19); 21.50 (CH_2-11); 23.01 (CH_2-16); 24.63 (CH_2-15); 26.11, 26.22 and 26.27 (3 × $(CH_3)_3$C); 31.73 (CH_3-21); 35.76 (CH-8); 36.62 (CH_2-10); 37.13 (C-7); 38.95 (CH_2-12); 44.27 (C-13); 46.16 (CH_2-1); 48.05 (CH-9); 57.12 (CH-14); 63.87 (CH-17); 68.13 (C-2); 103.95 (CH_2-4); 119.87 (CH-5); 140.50 (C-3); 149.62 (C-6); 209.53 (CO-20).

2α-Hydroxy-20-tert-butyldimethylsilyloxy-5α-pregn-20-ene-3,6-dione (**20**): white solid, m.p. 90–93 °C, R_F 0.35 (silica gel; chloroform–hexane–methanol 20:20:1). ^{1}H NMR: 0.18 (6 H, s, $(CH_3)_2$SiO); 0.67 (3 H, s, CH_3-18); 0.94 (9 H, s, $(CH_3)_3$CSi); 1.05 (3 H, s, CH_3-19); 1.21 and 1.75 (2 × 1 H, 2 × m, CH_2-15); 1.46 and 1.77 (2 × 1 H, 2 × m, CH_2-11); 1.26 (1 H, m, CH-14); 1.37 (1 H, m, CH-9); 1.76 and 2.02 (2 × 1 H, 2 × m, CH_2-16); 1.45 and 2.55 (2 × 1 H, 2 × m, CH_2-1); 1.48 and 2.08 (2 × 1 H, 2 × m, CH_2-12); 1.88 (1 H, m, CH-8); 2.03 and 2.40 (2 × 1 H, 2 × m, CH_2-7); 2.45 and 2.60 (2 × 1 H, 2 × m, CH_2-4); 2.62 (1 H, CH-17α); 2.70 (1 H, m, CH-5α); 3.48 (1 H, d, J = 3.3, OH); 4.03 and 4.10 (2 × 1 H, br s and d, J = 0.9, CH_2-21); 4.25 (1 H, m, CH-2β). ^{13}C NMR: –4.29 (($CH_3)_2$Si); 13.44 (CH_3-18); 14.12 (CH_3-19); 18.40 (SiC); 22.04 (CH_2-11); 24.37 (CH_2-16); 24.57 (CH_2-15); 26.07 (($CH_3)_3$C); 35.36 (CH_2-4); 38.01 (CH-8); 38.35 (CH_2-12); 42.96 (CH_2-10); 42.96 (C-13); 46.63 (C-7); 49.97 (CH_2-1); 54.04 (CH-9); 56.18 (CH-14); 56.96 (CH-5); 59.10 (CH-17); 72.51 (CH-2); 90.40 (CH_2-21); 159.41 (C-20); 208.11 (CO-6); 211.32 (CO-3).

2,3-Bis(*tert*-butyldimethylsilyloxy)-5α-pregn-2-ene-6,2-dione (**14**)

At r.t., 265 μl (1.2 mmol) of TBDMSiOTf were added to a solution of 3,6,20-trione **8** (20 mg, 58 μmol) in 1 ml of DCM and 175 μl (1.3 mmol) of triethyl amine stirred under argon atmosphere by a syringe through a rubber septum. After 1 h, additional 160 μl (1.2 mmol) of triethyl amine were added and the reaction mixture was treated with 1.2 mmol of TBDMSiOTf. The acidity of the reaction mixture was checked as follows: the reaction mixture was sampled by capillary on pH indicator paper and, after the evaporation of the solvent, the paper was moistened with water. Another 5-μl portion of TBDMSiOTf was added if necessary until the pH 3 to 5 was indicated on the paper. The reaction mixture was then stirred until TLC (aluminium oxide; hexane–ethyl acetate 10:1) revealed only traces of the starting compound **8**, R_F 0.1 and one major product with R_F 0.4, which strongly quenched UV_{254} fluorescence. For the workup, 10 ml of DCM were added and the reaction mixture was poured into 5 ml of a 10% water solution of sodium bicarbonate. The organic layer was separated and the water layer was washed with two 5 ml portions of DCM. The combined organic layers were dried by $MgSO_4$ and the solvent was evaporated. The crude product was purified by column chromatography on aluminium oxide (hexane–ethyl acetate 10:1). Enol **14** (22 mg, 66%) was obtained as white crystals after the evaporation of the solvents. After crystallization from diethyl ether, the m.p. was 180–182 °C.

Single Crystal X-ray Structure Analysis

The diffraction data of single crystals of **7** (colorless, 0.13 × 0.54 × 0.86 mm), **8** (colorless, 0.08 × 0.19 × 0.43 mm) and **15** (Fig. 2) (colorless, 0.12 × 0.13 × 0.85 mm) were collected on Xcalibur X-ray diffractometer with CuK_α (λ = 1.54180 Å) at 150 K. The structures were solved by direct methods with SIR92 [12] and refined by full-matrix least-squares on *F* with CRYSTALS [13]. The hydrogen atoms were all located in a difference map, but those attached to carbon atoms were repositioned geometrically. All of the hydrogen atoms were initially refined with soft restraints on the bond lengths and angles to regularize their geometry, after which the positions were refined with riding constraints. The other, non-hydrogen atoms were refined with anisotropic displacement parameters.

Crystal data for **7**: $C_{24}H_{36}O_4$, monoclinic, space group $P2_1$, a = 11.3272(6) Å, b = 7.5692(3) Å, c = 13.2246(7) Å, β = 111.279(6)°, V = 1056.54(10) Å^3, Z = 2, M = 388.55, 10467 reflections measured, 4284 independent reflections. Final R = 0.042, wR = 0.049, GOF = 1.052 for 4058 reflections with $I > 2\sigma(I)$ and 255 parameters.

Crystal data for **8**: $C_{21}H_{30}O_4$, monoclinic, space group $P2_1$, a = 7.3381(7) Å, b = 10.1698(8) Å, c = 12.2865(10) Å, β = 102.941(9)°, V = 893.62(13) Å^3, Z = 2, M = 346.47, 8563 reflections measured, 3328 independent reflections. Final R = 0.060, wR = 0.071, GOF = 0.997 for 2794 reflections with $I > 2\sigma(I)$ and 227 parameters.

Crystal data for **15**: $C_{27}H_{44}O_4Si$, monoclinic, space group $C2$, a = 33.535(4) Å, b = 6.6145(12) Å, c = 12.0723(17) Å, β = 91.202(10)°, V = 2677.3(7) Å^3, Z = 4, M = 460.73, 17625 reflections measured, 5250 independent reflections. Final R = 0.053, wR = 0.062, GOF = 1.042 for 4696 reflections with $I > 2\sigma(I)$ and 291 parameters.

CCDC 803973 (for **7**), 803974 (for **8**) and 803975 (for **15**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

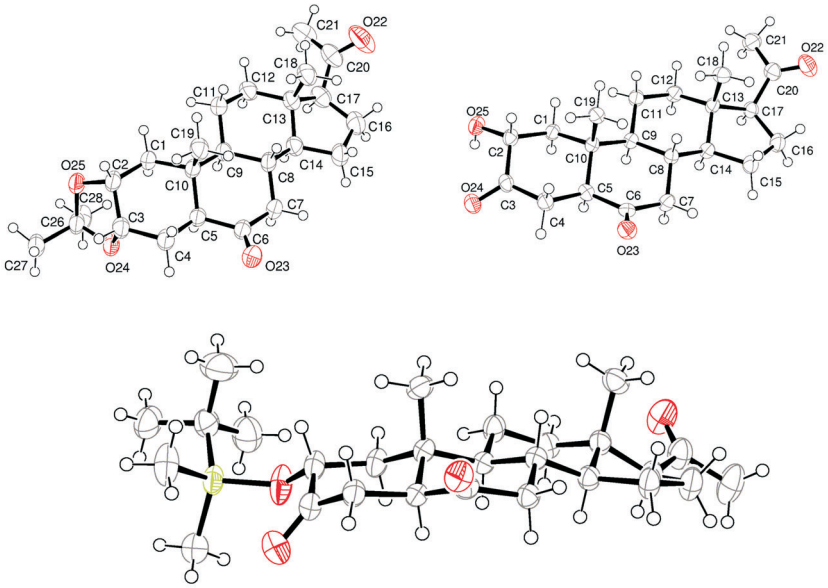


FIG. 2
ORTEP drawing **7**, **8** and **15**. Thermal ellipsoids are drawn at the 50% probability level

CONCLUSIONS

The attempts of the formation of a dimethyl dioxolene ring on the steroid skeletone were not successful. Notwithstanding that, we succeeded in finding the conditions for the transformation of the 2,3-diol group of common brassinosteroids to the 2,3-bis(*tert*-butyldimethylsilyloxy) enol group, a suitable precursor for regio- and stereoselective labeling of brassinosteroids by deuterium or tritium.

This work was performed as part of a research project of the Institute of Organic Chemistry and Biochemistry (Z40550506) and was supported by Grant IAA400550801 of the Grant Agency of the Academy of the Sciences of the Czech Republic.

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