Utility of *i*-Steroid-Route to Oxidized Sterol Bound to a Cross-linker: Synthesis of the Steroid Antigen

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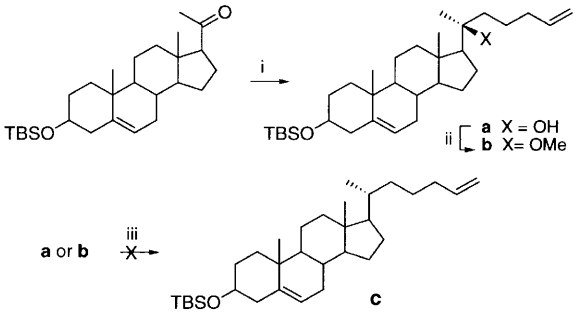
The target sterol, which was for preparation of oxidized sterol antigen to apply to a new antibody diagnostic method for circulatory disease, was successfully synthesized *via i*-steroid transformation as followings: 1) the Grignard reaction, 2) Barton-McCombie reaction, 3) regioselective photolytic-addition of thiolacetic acid toward 25-double bond, and 4) *in situ* Michael addition between the thiol and a cross-linker.

A major cause of atherosclerotic lesions in the arterial wall of human being would be cholesterol and cholesteryl ester.[1,2] Recently, one of the undeniable cause of atherosclerosis and familial hypercholesteremia would be low density lipoprotein (LDL)-cholesterol and its oxidized compounds,[3] such as 7-keto, 7α-, 7β-OH cholesterol. Thus, to develop a new antibody diagnostic and therapeutic method for these disease, the target oxidized sterol **14**[4] bound to a linker and the cross-linked protein **15** have been synthesized.

At first, we studied the route starting pregnenolone to reach the target **14** and examined to remove the tertiary hydroxy group (or methoxy group) at C-20 of the product in the Grignard reaction with pentenyl bromide (Scheme l).



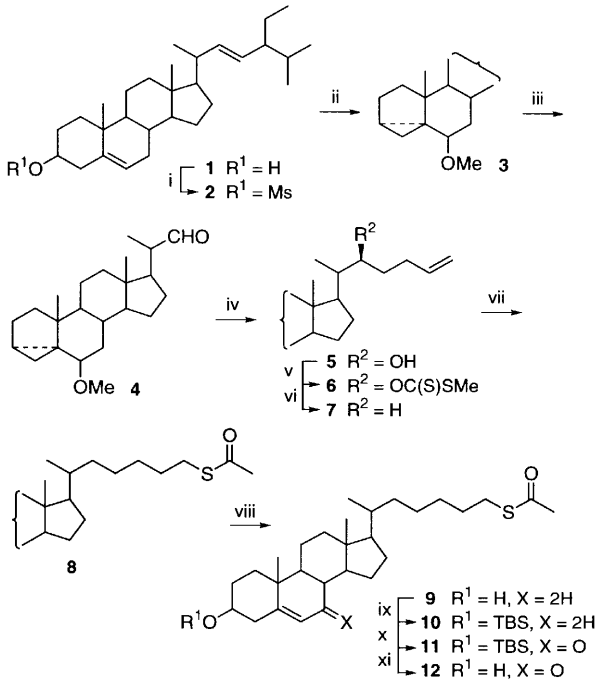
Scheme 1. Reagents and conditions: i, $H_2C=CH(CH_2)_3MgBr$, ether, –78 °C, 67%; ii, KH, MeI, THF, rt, 92%; iii, deoxygenation.[5–9]

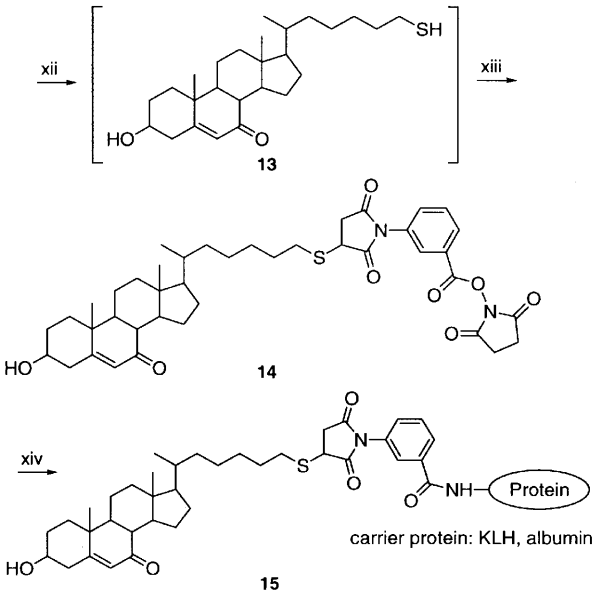
However, all attempts such as reaction with MX(NaI, LiBr)/TMSCl,[5] $ZnI_2/NaCNBH_3$,[6] $BF_3OEt_2/Et_3SiH/CH_3CN$,[7] tosylation or mesylation then the reaction with hydride,[8] and Barton-McCombie or its modified reaction,[9] etc., were found to be no useful, resulted in the elimination reaction mixtures, poor yields of the desired product **c**, or complete recovery of the starting material. Therefore, in this route we have completed the synthesis of the derivative of **14** having a extra methoxy substituent at C-20.

After several efforts of other routes, we tried to prepare the aldehyde from stigmasterol **1**,[10] however, in spite of the controlled-ozonolysis the yield is poor to give the complex reaction mixture, probably oxidized at the C-5 double bond. On the other hand, it is well known that a sterol is converted to *i*-steroid derivative by the solvolytic conditions.[10,11] The cyclopropane ring transformed is durable against ozonolysis, thereby this is a practical protection procedure of the double bond of the sterol system. Thus, the synthesis of the target **14** was started from this *i*-steroid and the obtained aldehyde **4** by the ozonolysis was used for the Grignard reaction (Scheme 2).

To introduce a side chain to the starting aldehyde **4** the Grignard reaction with butenyl bromide was carried out in dry ether at -78 °C to give the (22*S*)-alcohol **5** exclusively in 80% yield.[12] The deoxygenation of this secondary OH group was able to perform by the Barton-McCombie processes[9] as followings. 1) Quantitative preparation of xanthate **6** was achieved by treatment of the alcohol **5** with KH and CS_2 followed by addition of MeI at rt for 1 h. 2) The treatment of the xanthate **6** with *n*-Bu_3SnH in toluene gave the product **7** in excellent yield (98%), under reflux conditions in toluene for 30 min.

In the synthetic sequence, the key step to reach the target compound **15** is the introduction of a thiol functional group to **7**.[13] As the thiolacetic acid addition to olefin **7**, we have established a new photo-protocol. The irradiation of **7** in the presence of 1.1 equiv of thiolacetic acid with a high pressure mercury lamp for 4 h resulted in the formation of the thioester **8** in good yield (78%). The recovery from *i*-steroid **8** to 27-norcholesta-5,25-diene-3β-ol 9 was easily attained by the heating in





Scheme 2. Reagents and conditions: i, MsCl, Et_3N, CH_2Cl_2; ii, AcOK, MeOH; iii,O_3, Et_3N, CH_2Cl_2, **1**→**4**, overall yield, 44% (+acid product); iv, $H_2C{=}CH(CH_2)_2MgBr$, ether, –78 °C, 80%; v, KH, CS_2, MeI, THF, rt, 97%; vi, *n*-$BuSn_3H$, Toluene, Δ, 98%; vii, AcSH, CH_2Cl_2, *hν* (>300 nm), Pyrex tube, rt, 78%; viii, TsOH, dioxane:water = 9:3, 92%; ix, TBDMSCl, Im, DMF, rt, 97%; x, CrO_3, DMP, CH_2Cl_2, –25 °C, 70%; xi, THF:EtOH = 1:1, 36% HCl, rt, 95%; xii 5% KOH, H_2O-EtOH (1:1), then AcOH, rt; xiii, MBS, **11**→**14**, overall yield, 66%; xiv, KLH or albuin in buffer. All reactions were not optimized.

the presence of TsOH in dioxane-water.[11] After protection of **9** with TBDMSCl[14] the allylic oxidation of **10** by using CrO_3-3,5-dimethylpyrazole (DMP) complex in CH_2Cl_2 at ca. -30 °C gave the enone **11** in good yield (70%).[15] Subsequently, the deprotecting of compound **11** was performed by the controlled addition of HCl to give the corresponding alcohol **12**. Finally, the hydrolysis of thioester with 5% KOH solution (50% ethanol in water), acidification with acetic acid, and then *in situ* addition of MBS (*m*-maleimidobenzoyl-*N*-hydroxy-succinimide ester)[16] under nitrogen atmosphere successfully led to the target oxy-sterol **14** bound to the cross-linker in 66% yield. The protein **15** was prepared by the treatment of **14** with KLH (Keyhole Limpet Hemocyanin) or albumin in phosphate buffer solution in order to obtain monoclonal antibody specifically corresponding to the oxidized sterol.

We are continuing the synthesis of the other oxy-sterols such as 7α-, 7β-, 25-, 27-OH, and oxidized fatty sterols and the conjunction of the sterols with proper proteins for antigens and the production of antibodies of the oxy-sterols binding protein (KLH and serum albumin).

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References and Notes

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4 Typical spectral data of **14**: ^{1}H NMR ($CDCl_3$) δ 0.68 (s, 3H), 0.92 (d, *J* = 6.8 Hz, 3H), 1.20 (s, 3H), 2.70 (dd, *J* = 18.8, 3.6 Hz, 1H), 2.91 (bs, 4H), 3.34 (dd, *J* = 18.8, 9.2 Hz, 1H), 3.62-3.72 (m, 1H), 3.89 (dd, *J* = 9.2, 3.6 Hz, 1H), 5.68 (d, *J* = 1.6 Hz, 1H), 7.62-7.70 (m, 2H), 8.11-8.21 (m, 1H); FAB-HRMS ($[M+H]^+$) found 733.3524, calcd for $C_{41}H_{53}N_2O_8S$ 733.3523.
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