Synthesis of 6-Aryl-Substituted Cholesterol Derivatives from 3β-Acetoxy-6-iodocholest-5-ene and Arylboronic Acids Promoted by Palladium-Catalyzed Cross-Coupling

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Abstract: 3β-Acetoxy-6-iodocholest-5-ene was prepared with a convenient method from cholesterol and used as a key intermediate for the synthesis of 6-arylated cholesterol derivatives using a Suzuki–Miyaura cross-coupling. This versatile and efficient synthesis way can widely be used in the synthesis of other 6-aryl or 6-heteroaryl steroids as potential drugs.

Key words: cross-coupling, palladium, 6-aryl steroids, Suzuki reaction, 6-iodovinyl steroid

Cholesterol is most widely known for its association with arteriosclerotic heart disease; however, it is a necessary constituent of all our cells. Cholesterol plays a diverse array of functions in mammalian tissues. This sterol is a major lipid constituent of cellular membrane, and is involved in cell-signaling pathways and gene transcription.[1] A previous study attests to the vital roles of cholesterol in normal mammalian central nervous system development and function, and in a host of inherited and acquired neurological conditions.[2] Brain cholesterol is mainly produced in situ, and its metabolism is regulated independently of that in peripheral tissues.[3] Cholesterol is an essential precursor for steroid hormones and bile acids. A third group of its metabolites, the oxysterols, are oxidized forms of cholesterol.[4] These oxysterols, synthesized in the brain (called neurosterols) and in peripheral tissues, mediated feedback regulation of cholesterol biosynthesis rather than cholesterol itself.[5] Many studies have focused on the effects of oxysterols and showed diverse biological properties.[6]

Cholesterol is composed of three regions: a ring-structure region with four hydrocarbon rings (A, B, C, and D), a side chain on ring D, and a β-hydroxyl group. Oxidation in cells can occur on the ring B of the sterol, particularly on the C-7 position (7-ketocholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol), or on the side chain [25-hydroxycholesterol, 20(*S*)-hydroxycholesterol, 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol, 27-hydroxycholesterol].[7] The double bond Δ5–6 can be also the target of free radical attacks (5,6α- and 5,6β-epoxycholestanol).[8] These structures showed that the 5-, 6-, and 7-positions in the ring B are the most sensitive sites for reactions involved in various biological mechanisms.[9]

By analogy to oxysterols, we have developed in previous studies, a series of aminosterols which have demonstrated promising activities.[10] There is an increasing interesting development of new strategies to introduce other groups into specific positions of steroidal nuclei to modify their biological properties. Two strategies have been described in the literature. The first is the introduction of an heteroatom on the steroidal skeleton,[11] and the second is the functionalization of the steroid which was used of various steroids with potential therapeutic applications.[12]

A series of 17-pyridyl-androstene was prepared from 3β-acetoxy-17-triflyloxy-5α-androst-16-ene (2-, 3-, and 4-pyridyl) as inhibitors of human cytochrome $P450_{17\alpha}$ (17α-hydroxylas-C17,20-lyase), which is a key enzyme in androgen hormone biosynthesis.[13] Some other heterocyclic groups were introduced to the C-17 position of 3β-hydroxy-androsta-5,16-diene.[14] Herein we focused on the incorporation of aryl substituent on the C-6 position. However, to the best of our knowledge, the ring B of steroids has received little attention, and it was also noticed that few aryl or heterocyclic groups were introduced in this ring via C–C coupling reaction. The coupling of 17-acetoxy-6-chloro-pregna-4,6-diene-3,20-dione (commercial starting material) and arylboronic acids was carried out by the Suzuki–Miyaura cross-coupling reaction.[15]

As part of our ongoing medicinal chemistry program, the aim of our project is based on the introduction of an aryl group on the C-6 position of 3β-acetoxycholest-5-ene skeleton. The main goal of our work is to develop a convenient synthetic method of 3β-acetoxy-6-iodocholest-5-ene, used as a key intermediate, which is explored as a building block for the synthesis of 6-arylated cholesterol derivatives by Suzuki–Miyaura cross-coupling reaction (Scheme 1).

In the literature and to our knowledge, 6-iodocholest-5-en-3β-ol has been described in one paper from cholesterol in four steps via 6-acetoxymercuricholest-5-ene intermediate. However, this method leads to a mixture of compounds and that proceeds with low yields (2–20%).[16] Furthermore, the high toxicity of organomercuric compounds has been observed and widely studied.[17]

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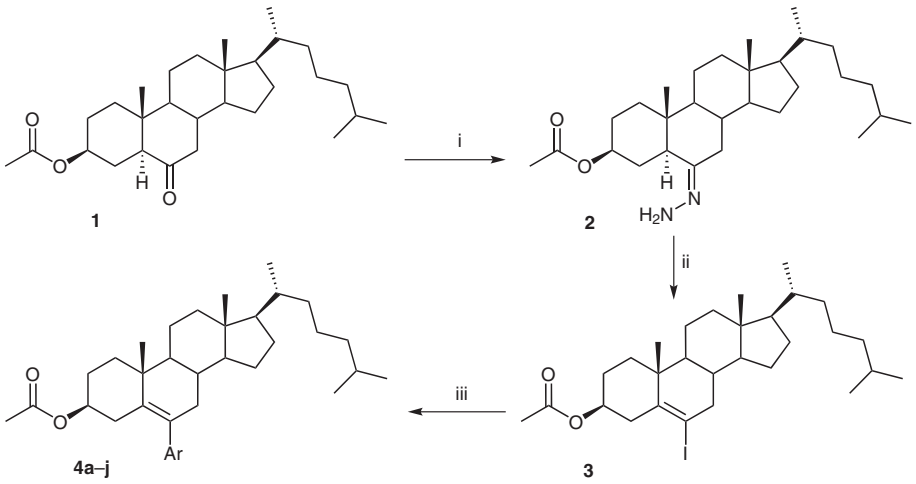


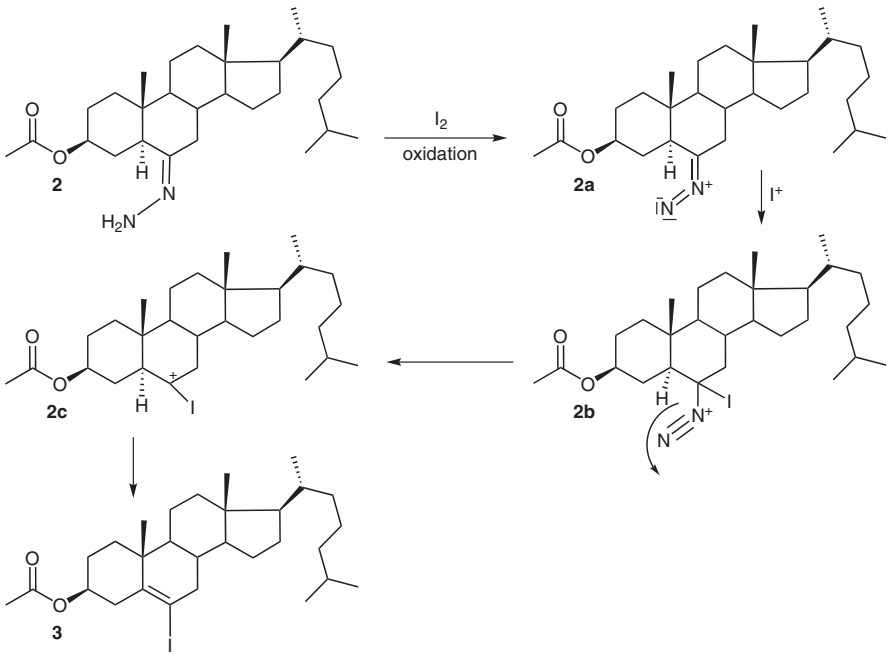
Scheme 1 *Reagents and conditions*: (i) $N_2H_4 \cdot xH_2O$, AcOH, EtOH, reflux, 12 h; (ii) I_2, 1,1,3,3-tetramethylguanidine, anhyd THF, 50 °C, 60 h; (iii) aryl boronic acid, $Pd(OAc)_2$, Ph_3P, K_2CO_3, DMF, 150 °C, 12–18 h.

For these reasons, this method cannot be applicable to the development of biological compounds. On the other hand, an improved preparation of vinyl iodide on the C-17 position of the steroid skeleton was described by Barton.[18] We explore here this method for the synthesis of 3β-acetoxy-6-iodocholest-5-ene (**3**) which was prepared as depicted in Scheme 1.

3β-Acetoxy-5α-cholestan-6-one (**1**) has been prepared from cholesterol according to our reported procedure.[10b] The hydrazone derivative **2** has been obtained in a good yield from the latter ketone **1**, using hydrazine hydrate and acetic acid in ethanol.[19] This hydrazone **2** was then oxidized by iodine in the presence of tetramethylguanidine base leading to 3β-acetoxy-6-iodocholest-5-ene (**3**).[20] The mechanism proposed for the transformation of hydrazone to iodovinyl is indicated in Scheme 2.

The hydrazone **2** is oxidized by iodine to the diazo compound **2a** which reacts further to give the iodo derivative **2b**. A loss of nitrogen leads to the key iodo carbocation **2c**. Elimination of proton on C-5 position of steroid skeleton gives vinyl iodide **3**.

The second goal of our work is to prepare the 6-aryl-substituted cholesteryl acetate **4a–j** by palladium-mediated reaction of 3β-acetoxy-6-iodocholest-5-ene (**3**) with various arylboronic acids. Though the Suzuki–Miyaura coupling is a universal and widely employed tool in organic synthesis, a protocol adjustment is often needed to obtain the optimal performance with the catalytic system. The step reaction iii (Scheme 1) has been improved by exploring various reagents and conditions (Table 1). The progress of the reaction was followed by thin-layer chromatography. These conditions have also been carried out



Scheme 2 Mechanism proposed of ketone hydrazone oxidation by iodine

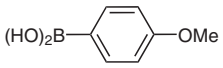
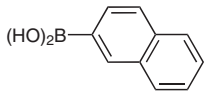
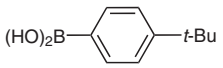
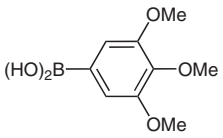
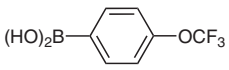
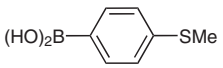
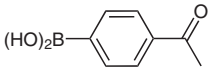
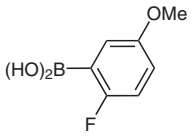
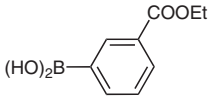
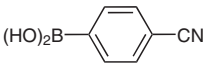
Table 1 Optimization of Suzuki Coupling Conditions with *p*-Methoxyphenylboronic Acid

Catalyst	Base	Solvent	Temp (°C)	Time (h)	Yield (%)
$Pd(PPh_3)_2Cl_2$	CuI, DIPA	DME	70	28	–
$Pd(PPh_3)_2Cl_2$	K_2CO_3	dioxane–H_2O (3:1)	100	24	–
$Pd(OAc)_2$, $P((OMe)_3Ph)_3$	K_2CO_3	DMF	150	24	<10
$Pd(OAc)_2$, $P((OMe)_3Ph)_3$	CsF, CuI	DMF	150	24	<10
$Pd(OAc)_2$, Ph_3P	K_2CO_3	DMF	150	12	50

under microwave irradiation but none improvement in yield reactions was achieved.

The best conditions found were palladium acetate, triphenylphosphine, and potassium carbonate in DMF with conventional heating (12–18 h). Results are presented in Table 2.

Table 2 Yields of 3β-Acetoxy-6-arylcholest-5-ene[21]

Arylboronic acid	Time (h)	Product **4a–j**	Yield (%)
	12	**4a**	50
	12	**4b**	50
	12	**4c**	42
	18	**4d**	67
	18	**4e**	50
	18	**4f**	46
	18	**4g**	79
	18	**4h**	64
	18	**4i**	62
	15	**4j**	29

Our method has been applied to diverse arylboronic acids, which have various functions (such as protected alcohol, ester, cyano group, ketone, sulfur product, and trifluoromethoxy group) with correct yields.

In summary, we have reported a convenient synthetic method of 3β-acetoxy-6-iodocholest-5-ene as a key intermediate for C–C cross-coupling reaction. This versatile method can be used to prepare other various 6-iodovinylsteroids, which were coupled with various aryl or heteroaryl boronic acids in order to prepare scaffolds for potent chemical drugs libraries.

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(19) **Synthesis of 3β-Acetoxy-5α-cholestane-6-hydrazone (2)**
Hydrazine hydrate (0.42 mL, 13.5 mmol) was added to a solution of 3β-acetoxy-5α-cholestan-6-one (**1**, 3.00 g, 6.8 mmol) and AcOH (0.01 mL, 0.2 mmol) in EtOH (70 mL). The mixture was refluxed for 24 h. The reaction mixture was concentrated, diluted with H_2O (70 mL) and then extracted with CH_2Cl_2 (4 × 30 mL). The combined CH_2Cl_2 extracts were dried over $MgSO_4$ and evaporated under reduced pressure to give compound **2** as a white powder (3.06 g, 99%); mp 56 °C. IR (KBr): ν = 3480–3300 (NH_2 hydrazone), 2950–2875 (CH alkane), 1727 (C=O ester), 1651 (C=N) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.96 (s, 2 H, NH_2 D_2O exchange), 4.69–4.66 (m, 1 H, C_3H), 2.92–2.97 (dd, 1 H, *J* = 13.28, 4.48 Hz, C_5H), 2.04–2.10 (each 1 H, m, C7αH and 7β-CH), 2.06 (s, 3 H,CH_3COO), 0.92–0.89 (m, 3 H, 19-Me), 0.87 and 0.86 (each 3 H, d, *J* = 1.96 Hz, 26-Me and 27-Me), 0.63 (s, 3 H, 18-Me) ppm.^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6 (CH_3COO), 154.1 (C-6), 73.7 (C-3), 56.4 (C-14), 56.1 (C-17), 50.5 (C-9), 48.8 (C-13), 42.9 (C-5), 39.4 (C-12), 38.8 (C-10), 36.0 (C-1, C-20, C-22), 35.6 (C-7, C-8), 29.3 (C-4), 28.0 (C-16, C-25), 28.9 (C-2), 24.0 (C-19), 23.7 (C-15, C-23), 22.7 (C-26), 22.5 (C-27), 21.3 (C-11), 21.3 (CH_3COO), 19.6 (C-21), 11.9 (C-18) ppm.

(20) **Synthesis of 3β-Acetoxy-6-iodocholest-5-ene (3)**
A 100 mL round-bottomed flask equipped with a magnetic stirring bar was charged with iodine (4.76 g, 18.8 mmol) and dry THF (5 mL) and flushed with nitrogen. The reactor was pushed into an ice bath (0 °C), and then 1,1,3,3-tetramethylguanidine (6 mL, 46.9 mmol) was added. Compound **5** was then dissolved into dry THF (30 mL) and added dropwise, and the solution was kept under stirring at 0 °C for 2 h. The mixture was filtered and the solvent evaporated. The solution was then stirred at 50 °C for 48 h. The reaction mixture was dissolved in Et_2O, washed with 1 N HCl, 5% $NaHCO_3$, sat. $Na_2S_2O_3$, H_2O, and dried over $MgSO_4$. The crude product was purified by column chromatography (silica gel, cyclohexane–EtOAc = 9:1) to give the product as a yellow oil (4.55 g, 88%). IR (KBr): ν = 2935 (CH alkane), 1735 (C=O ester), 1030 (C=CI) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.64–4.58 (m, 1 H, C_3H), 2.94 and 2.93 (each 1 H, m, $C_{4\alpha}H$ and $C_{4\beta}H$), 2.63 and 2.60 (each 1 H, m, $C_{7\alpha}H$ and $C_{7\beta}H$), 2.05 (s, 3 H, CH_3COO), 1.07 (s, 3 H, 19-Me), 0.91 (d, *J* = 6.83 Hz, 3 H, 21-Me), 0.87 and 0.85 (each 3 H, d, *J* = 1.96 Hz, 26-Me and 27-Me), 0.66 (s, 3 H, 18-Me) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.3 (CH_3COO), 142.8 (C-5), 101.3 (C-6), 72.7 (C-3), 55.9 (C-14, C-17), 49.6 (C-9), 42.4 (C-13), 41.2 (C-12), 39.5 (C-7, C-24), 36.8 (C-4), 36.1 (C-1), 35.7 (C-22), 35.3 (C-20), 34.4 (C-10), 28.2 (C-16), 27.9 (C-25), 27.7 (C-2), 24.1 (C-15), 23.8 (C-23), 22.8 (C-27), 22.5 (C-26), 21.4 (C-11), 21.2 (CH_3COO), 19.7 (C-19), 18.7 (C-21), 11.8 (C-18) ppm.

(21) **General Procedure for the Coupling of 6-Iodovinylsteroid with Boronic Acids**
In a vial with a screw cap, compound **3** (0.30 g, 0.5 mmol), boronic acid (1.1 mmol), $Pd(OAc)_2$ (0.01 g, 0.05 mmol), Ph_3P (0.03 g, 0.1 mmol), and K_2CO_3 (0.19 g, 1.4 mmol) were mixed in DMF (6 mL) under Ar atmosphere. The mixture was stirred at 150 °C for 12–18 h, then diluted with H_2O (30 mL), and extracted with Et_2O (4 × 15 mL). The combined organic layers were dried over $MgSO_4$. The crude product was purified by column chromatography on deactivated alumina (6% H_2O), eluting by cyclohexane–EtOAc (99:1).
3β-Acetoxy-6-(4-methoxy)phenylcholest-5-ene (4a): White amorphous solid. Yield: 48 mg (50%). IR (KBr): ν = 2928 (CH alkane), 1716 (C=O ester), 1607–1509 (C=C) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.01 (d, 2 H, *J* = 8.79 Hz, H-ar), 6.85 (d, 2 H, *J* = 7.79 Hz, H-ar),4.62–4.50 (m, 1 H, C_3H), 3.80 (s, 3 H, OCH_3), 2.54 and 2.50 (each 1 H, m, $C_{4\alpha}H$ and $C_{4\beta}H$), 2.20 and 2.16 (each 1 H, m, $C_{7\alpha}H$ and $C_{7\beta}H$), 1.95 (s, 3 H, CH_3COO), 1.10 (s, 3 H, 19-Me), 0.92 (d, 3 H, *J* = 6.83 Hz, 21-Me), 0.87 and 0.85 (each 3 H, d, *J* = 1.96 Hz, 26-Me and 27-Me), 0.71 (s, 3 H, 18-Me) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4 (CH_3COO), 157.9 (C-4ar), 136.4 (C-6), 134.7 (C-5), 133.6 (C-1-ar), 129.2 (C-2 and C-2′-ar), 113.5 (C3 and C-3′-ar), 73.9 (C-3), 56.6 (C-14), 56.1 (C-17), 55.2 (OCH_3), 49.9 (C-9), 42.3 (C-13), 39.8 (C-24), 39.6 (C-12), 39.5 (C-1), 37.3 (C-22), 36.9 (C-20), 36.2 (C-7), 35.8 (C-10), 32.8 (C-8), 32.0 (C-4), 28.3 (C-16), 27.9 (C-25), 27.7 (C-2), 24.2 (C-15), 23.8 (C-23), 22.8 (C-26 and C27), 21.4 (C-11), 21.1 (CH_3COO-), 19.5 (C-19), 18.7 (C-21), 11.9 (C-18). MS (30eV, IE): *m/z* = 534.3 (8) [M^+], 474.3 (100) [$M^+ - CH_3COOH$], 459.3 (8), 368.3 (25). Anal. Calcd for $C_{36}H_{54}O_3$ (534.81): C, 80.85; H, 10.18. Found: C, 80.42; H, 9.77.