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A Convenient and Highly Stereoselective Synthesis of 4α-Deuterio and -Tritio Steroids Catalyzed by Pd

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Abstract: *A simple and highly stereoselective synthesis of [4α-^{2}H]- and [4α-^{3}H]-Δ^{5}-3β-hydroxysteroids is presented. Palladium(0)-mediated borodeuteride reduction of readily-available cholest-5-en-3β,4β-diol cyclic carbonate provides [4α-^{2}H]- Δ^{5}-and Δ^{4}-cholesterol in a 12:1 ratio. Reduction of [4α-^{3}H]-cholest-5-ene-3β,4β-diol cyclic carbonate with NaB^{1}H$_{4}$ and Pd(0) resulted in [4β-^{3}H]-cholesterol.*

Chemical studies of the biosynthesis of natural products have frequently relied upon the employment of regio- and stereospecifically isotopically-substituted natural compounds to aid in the elucidation of metabolic pathways.[1] The fate of a particular labile atom during the metabolism of a biological compound has often been ascertained in this way. In addition, isotopic substitution of atoms which are known to be non-labile also provides information on the metabolic fate of the molecule as a whole.

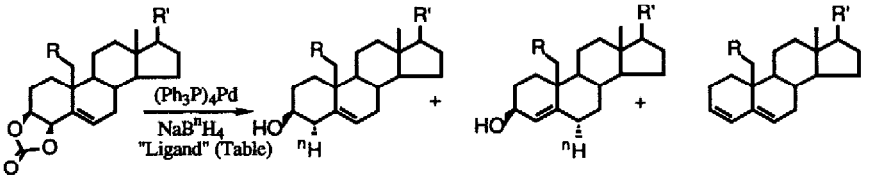
In the course of our studies on the biosynthesis of 19-nor sterols in the marine sponge *Axinella polypoides*, it became necessary to determine the fate of sterol hydrogen atoms in the 4α and 4β positions during the biological conversion of dietary cholesterol (**2a**) into 19-nor-5α-cholestan-3β-ol.[2]

4β-Deuterio-, and 4β-tritio-cholesterols are well known and are readily available from cholesterol through simple transformations.[3] Their 4α analogs (**2b** and **2c**) have also been reported,[4] but the synthesis used has subseqently been shown to be invalid.[5] Indeed, we have independently verified that borohydride reduction of 4-ketocholesteryl benzoate gives less than 7% of the desired 4β-alcohol, the key intermediate in the reported preparation of 4α-deuterio and -tritio steroids. The major product of the reduction, the 4α alcohol, is not transformed to cholesterol under the reported conditions.[4] Only one other protocol for the preparation of [4α-^{n}H]-Δ^{5} steroids has been published,[6] and it is not readily applicable for tritiation.

This paper reports a new procedure for the stereospecific introduction of deuterium or tritium into the 4α position of Δ^{5} steroids based on the pioneering work of Hutchins et al.,[7a] and Jones and Knox.[7b]

Reduction of cholest-5-ene-3β,4β-diol cyclic carbonate[8] (**1**) with sodium borohydride in the presence of 10 mole% tetrakis(triphenylphosphine)palladium(0) provides 82% of a 25:1 mixture of cholesterol, **2a**, and Δ^{4}-cholesterol, **3a** (Table, entry A). Also produced is cholesta-3,5-diene (**4**) in 3-16% yield (MS = 368.3(2.5%), 313.0(2.5), 256.3(12.5), 207.1(100), 185.1(25), 129.1(87.5), 109.1(35), 61.0(100), 55.1(82.5); ^{1}H nmr (200 MHz) δ 0.697 (s;3H), 0.858 (d;6H;J=6.6Hz), 0.914 (d,3H,J=6.5Hz), 0.946 (s;3H), 5.39 (bs;1H), 5.60 (m;1H), 5.924 (bd,1H,J=9.2Hz)) which presumably arises from carbonate elimination in the intermediate π-

allylpalladium species (submission of the other reaction products to the reaction conditions fails to produce this diene). The 3β,4β-diol resulting from phosphine-promoted cleavage of the starting material was also present in the product mixture. The analogous reaction with sodium borodeuteride resulted in a 12:1 mixture of [4α-^{2}H]-cholesterol,[9a] **2b**, and [6α-^{2}H]-Δ^4-cholesterol, **3b**[9b] (Table, entry E). This protocol was also used for the 4α-deuteration and tritiation of steroid carbonates **5**[10a] and **6**[10b] to provide Δ^5-sterols **7b**[9c] and **8b**.[9d]



1 R=H; R'=C_8H_{17} **2** R=H; R'=C_8H_{17} **3** R=H; R'=C_8H_{17} **4** R=H; R'=C_8H_{17}
5 R=H; R'=OTBS **7** R=H; R'=OTBS
6 R=OTMS; R'=C_8H_{17} **8** R=OTMS; R'=C_8H_{17}

a: n=1; **b**: n=2; **c**: n=3

Table. Reduction of Steroidal Carbonate **1**.

	Conditions			Product Yields[a]				
Entry	Temp.	Ligand	Reducing Agent	2	3	4	3β,4β-Diol	Recovered Starting Material
A	RT	Ph_3P	NaB^1H_4	79% (25)	3% (1)	9%	9%	0
B	RT	Ph_3P	NaB^2H_4	19% (9)	2% (1)	6.8%	45%	27%
C	RT	dppe	NaB^1H_4	61% (6.5)	9% (1)	4%	26%	0
D	RT	dppe	NaB^2H_4	64% (5.3)	12% (1)	8.2%	16%	0
E	70°C	Ph_3P	NaB^2H_4	58% (12)	5% (1)	16%	21%	0
F	70°C	dppe	NaB^2H_4	64% (3.9)	16% (1)	3%	16%	0

[a] As determined by 400 MHz ^{1}H nmr. Numbers in parenthesis refer to product ratios.

In a typical reaction,[7a] **1**[8] (1 eq.) was added via syringe to a stirred solution of $(Ph_3P)_4Pd$ (0.1 eq.), and Ph_3P (0.7 eq.) in THF under argon (final concentration of substrate = 0.05 M). Sodium borodeuteride (2 eq) was added to the reaction mixture under argon then stirred at 75° C under Ar. Reaction was usually complete after 2-3 h. The Table shows product yields and Δ^5:Δ^4 product ratios for varying conditions of temperature, added ligand, and reducing agent.

The most notable finding is that there is a pronounced deuterium-isotope effect. With sodium borohydride as the reducing agent, the reaction is complete at room temperature in 2-3 hours. Upon reduction of **1** with 80 equivalents of a 1:1 mixture of NaB^1H_4 and NaB^2H_4, a 2.3:1 ratio of cholesterol to deuteriocholesterol was obtained. After reduction of **1** with sodium borotritide, steroidal alcohol **2c** had a

specific activity of 120 mCi/mmole-^{3}H whereas that of the reagent was 350 mCi/mmole-^{3}H, indicating a tritium content that is almost one third that of the borotritide reagent. It is possible that the rate-limiting step for this reaction involves either the formation or reductive elimination of a π-allylpalladium(II) hydride intermediate.[11]

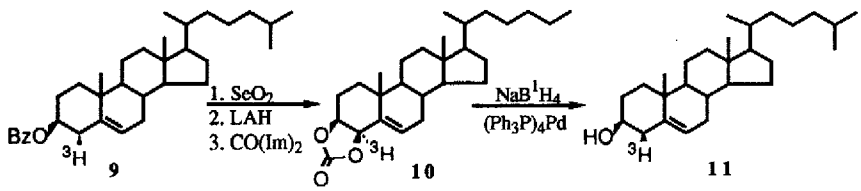
This reaction could also be performed with the generation of $(Ph_3P)_4Pd$ *in situ* through a premixing of $Pd_2(dba)_3CHCl_3$[12] and Ph_3P. While this method provides slighly higher yields it is incompatable for tritiation as the borotritide reagent is wasted on the reduction of dibenzylideneacetone.

That this reaction does not involve palladium-mediated oxidation[13] at C-3 followed by borohydride reduction of the resulting ketone is suggested by the fact that no deuterium at C-3 was observed in the ^{2}H nmr spectrum of **2b** and also because the ^{1}H nmr spectrum of the product mixture showed no evidence of the presence of 3α-hydroxy cholesterol.

Determination of Stereochemical Purity

Both 400 MHz ^{1}H nmr as well as ^{2}H nmr fail to resolve the 4α and 4β protons in **2b** sufficiently to allow for quantitation of deuterium incorporation. Oxidation of the benzoate of [4α-^{2}H]-cholesterol (^{1}H nmr 400 MHz: δ = 2.22 ppm; m; 1.13 H; C-4 protons) with SeO_2, which has been shown to provide 4β-hydroxy steroids without randomization of the hydrogen atoms at C-4,[4b] provided [4α-^{2}H]-cholest-5-ene-3β,4β-diol 3-benzoate whose ^{1}H nmr spectrum showed a 4α-proton resonance (δ = 4.39 ppm; bd; J = 2.3 Hz) with an area equal to 0.12 protons, indicating a stereospecificity of deterium introduction of at least 99%.

This procedure was also used for the synthesis of [4β-^{3}H]-cholesterol. Oxidation of the benzoate of **2a** (**9**, specific activity = 28 mCi/mmol) with SeO_2[4b] resulted in [4α-^{3}H]-4β-hydroxycholest-5-en-3β-yl benzoate (specific activity = 26 mCi/mmol). Conversion to [4α-^{3}H]-cholest-5-ene-3β,4β-diol cyclic carbonate (**10**) was accomplished by reduction with LAH and treatment with carbonyldiimidazole. Reduction of this carbonate with NaB^1H_4 in the presence of 10 mol% $(Ph_3P)_4Pd$, and Ph_3P at 70°C produced a 5:1 mixture of [4β-^{3}H]-cholesterol (**11**) and [4-^{3}H]-Δ^4-cholesterol in 84% radiochemical yield which were readily purified by reverse phase HPLC. Oxidation of **11** with SeO_2[4b] resulted in the complete loss of tritium.



[4α-^{n}H] Δ^5-3β-Hydroxy steroids can also be converted to their [4-^{n}H]-Δ^4-3-keto steroid analogs by oxidation with PCC followed by treatment with dilute ethereal HCl at 0°C without loss of the isotopic label.[14] Due to the known lability of the steroidal 4β hydrogen atom during many biological transformations that involve Δ^5 to Δ^4 isomerization (such as the biosynthesis of testosterone,[15] progesterone,[16] and corticosteroid[16]) this procedure may prove to be useful as a convenient general method for precursor labelling in isotope tracer experiments.

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9. a. **2b**: ^{1}H nmr (400 MHz) δ 0.67 (s,3H), 1.00 (s,3H), 2.21 (bd,J=10.4Hz,1H), 3.52 (ddd,J-11.2,11.2,4.1Hz,1H), 5.35 (dd,J=3.2,2.0Hz,1H); HRMS calcd for $C_{27}H_{45}O^2H$ 387.3611, found 387.3630; ^{2}H nmr (61.4 MHz) δ 2.27 ppm, (cf. [4β-^{2}H]-cholesterol[3] δ 2.22 ppm).
   b. **3b**: ^{1}H nmr (400 MHz) δ 0.673 (s,3H), 0.853 (d,3H,J=9.2Hz), 0.861 (d,3H,J=6.4Hz), 0.897 (d,3H,J=6.4Hz), 1.045 (s,3H), 2.17 (bd,1H,J=18Hz), 4.15 (m,1H); 5.269 (d,1H,J=1.6Hz); HRMS calcd 387.3611, found 387.3597. MS: 387(9.6%), 369(31.2), 317(24.1), 204(19.5), 162(21.9), 161(21.2), 147(45.8), 135(40.2), 107(100).
   c. **7b**: ^{1}H nmr (200 MHz) δ -0.001 (s,3H), 0.007 (s,3H), 0.710(s,3H), 0.873 (s,9H), 1.011(s,3H), 2.214 (bd,1H,J=12.8Hz), 3.52 (m,1H), 3.546 (t,J=8,2Hz), 5.34 (m,1H); HRMS calcd for $C_{25}H_{43}O_2{}^2HSi$ 405.3174, found 405.3133.
   d. **8b**: ^{1}H nmr (400 MHz) δ 0.072 (s, 9H, Me_3Si), 0.699 (s, 3H, 18-Me), 0.857 (d, 3H, J=6.6, 26-Me), 0.861 (d, 3H, J=6.6, 27-Me), 0.909 (d, 3H, J=6.5, 21-Me), 2.19 (m, 1.10H, 4-H_2), 3.521 (d, 1H, J=10.6, 19-H), 3.55 (m, 1H, 3-H), 3.761 (d, 1H, J=10.6, 19-H'), 5.58 (m, 1H, 6-H); HRMS calcd for $C_{30}H_{51}{}^2HOSi$ (M^+-H_2O) 457.3850, found 457.3849.
10. a. Compound **5** was prepared from androst-5-ene-3β,17β-diol 17-*t*-butyldimethylsilyl ether 3-benzoate by SeO_2 oxidation to provide androst-5-ene-3β,4β,17β-triol 17-*t*-butyldimethylsilyl ether 3-benzoate (mp=173-174°C) in 52% yield which was reduced with LAH to give a diol. Heating in toluene with carbonyldiimidazole provided androst-5-ene-3β,4β,17β-triol 17-*t*-butyldimethylsilyl ether 3,4-cyclic carbonate in 75% yield for the two steps (mp = 205-206°C): ^{1}H nmr 400 MHz: δ -0.002 (s,3H), 0.004 (s,3H), 0.730 (s,3H), 0.872 (s,9H), 1.123(s,3H), 2.199 (dt,1H,J=18,4.8Hz), 3.553 (t,1H,J=8.2), 4.688(q,1H,J=13,7.0Hz), 4.937 (dd,1H,J=7.1,1.8Hz), 5.912 (dd,1H,J=4.5,2.3Hz); ^{13}C nmr (75 MHz): δ -5.1, -4.8, 11.0, 17.8, 20.3, 21.7, 23.2, 24.3, 25.6, 30.3, 30.7, 31.3, 31.5, 35.9, 36.8, 43.1, 48.0, 51.1, 76.3, 81.6, 82.7, 134.8, 135.5, 201.0; HRMS calcd for $C_{22}H_{33}O_4Si$ (M^+ -C_4H_9) 389.2148, found 389.2136.
    b. Compound **6** was prepared from 19-hydroxycholesterol by SeO_2 oxidation to provide cholest-5-ene-3β,4β,19-triol in 42% yield (mp 182-183°C): ^{1}H nmr (400 MHz) δ 0.694 (s,3H), 0.853 (d,3H,J=6.6Hz), 0.858 (d,3H,J=6.6Hz), 0.899 (d,3H,J=6.5Hz), 2.162 (ddd,1H,J=18.6,5.8,4.7Hz), 3.62 (m,1H), 3.693 (d,1H,J=10.8Hz), 3.817 (d,1H, J=10.8Hz), 4.12 (dd,1H,J=3.4,1.1Hz), 5.952 (dd,1H, J=4.7,2.6Hz); ^{13}C nmr (100 MHz): δ 12.0, 18.7, 21.2, 22.5, 22.8, 23.8, 24.1, 26.2, 28.0, 28.2, 31.8, 32.5, 33.9, 35.7, 36.1, 39.5, 39.8, 40.7, 42.3, 50.4, 56.0, 57.6, 66.1, 71.9, 76.5, 133.1, 137.9; HRMS calcd for $C_{27}H_{44}O_2$ (M^+-H_2O) 400.3341, found 400.3349. The triol was converted to **6** by reaction with carbonyldiimidazole followed by mild aqueous hydrolysis with dilute KOH to provide cholest-5-ene-3β,4β,19-triol 3,4-cyclic carbonate in 94% yield from the triol: ^{1}H nmr (200 MHz) δ 0.743 (s,1H), 0.851 (d,6H,J=6,6Hz), 0.904 (d,3H,J=6.5Hz), 3.708 (d,2H,J=2.3Hz), 4.80 (m,1H), 5.025(d,1H,J= 7.7Hz), 6.191 (dd,1H,J=4.1,2.8Hz); HRMS calcd for $C_{28}H_{44}O_4$ 444.3240, found 444.3255. Silylation with N,O-bis(trimethylsilyl)acetamide in refluxing $CHCl_3$ produced **6** in 90% isolated yield: ^{1}H nmr (200 MHz) δ 0.072 (s,9H), 0.719 (s,3H), 0.855 (d,6H,J=6.6Hz), 0.907 (d,3H,J=6.4Hz), 3.623 (dd,2H,J=14.7,10.7), 4.75 (m,1H), 5.485 (d,1H,J=7.6Hz), 6.06 (m,1H); ^{13}C nmr (50 MHz) δ -0.09, 11.8, 18.4, 21.3, 22.4, 22.6, 23.6, 23.9, 24.3, 24.7, 27.8, 28.1, 31.3, 31.8, 35.6, 36.0, 39.4, 39.9, 40.8, 42.5, 47.5, 55.9, 57.4, 64.9, 76.5, 82.7, 131.3, 137.7 (OCO-O unobserved)
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