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Stereocontrolled Syntheses of 24(*S*),25-Epoxycholesterol and Related Oxysterols For Studies On the Activation of LXR Receptors

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Abstract: *Efficient syntheses are described of desmosterol (**4**), the corresponding 24(S),25 epoxide (**6**) and various analogs of **6** for evaluation as ligands and functional activators of LXR receptors.*

A number of oxysterols can bind the nuclear proteins LXR and PXR, orphan members of the nuclear receptor superfamily.[1,2] Activation of LXRα triggers gene transcription and up-regulation of cholesterol 7α-hydroxylase (Cyp7a), the rate-limiting enzyme for bile acid synthesis, and other lipid-metabolizing genes.[3] LXRα and LXRβ were shown to be significantly activated by various cholesterol derivatives bearing oxygen on the aliphatic sidechain, such as 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol, and 24(*S*),25-epoxycholesterol (24(*S*),25-ECH).[4,5] Since 24(*S*),25-ECH is formed during normal cholesterol biosynthesis from squalene 2,3(*S*), 22(*S*),23-dioxide via 24(*S*),25-oxidolanosterol,[6,7] and since this molecule can serve to clock cholesterol biosynthesis, it is a logical candidate for a significant role in LXR activation. Despite the potential importance of 24(*S*), 25-ECH and its possible role also in the regulation of HMG-CoA reductase activity,[8] an efficient stereocontrolled synthesis has been lacking.[9,10] Described herein is an effective synthesis of desmosterol (**4**), its stereocontrolled conversion to 24(*S*),25-ECH (**6**) and the synthesis of various analogs of **6** which are of interest in connection with understanding the nature of oxysterol ligand binding to LXR and structure-activity relationships for LXR activation.[11]

A practical synthetic route to desmosterol and 24(*S*),25-ECH (**6**) from a readily available starting material is outlined in Scheme 1. Reduction of ester **1**[12] to the alcohol, Swern oxidation to aldehyde **2** and Horner-Emmons condensation with trimethylphosphonoacetate[13] produced the expected *E*-α,β-unsaturated ester which by reduction with magnesium and methanol afforded methyl 3β-hydroxycholenate **3**. Reduction of **3** to the aldehyde, Wittig olefination, and desilylation gave desmosterol (**4**) in quantity. This route also allows access to several oxysterol analogs of interest as potential ligands of LXRs.[14] Sharpless asymmetric dihydroxylation of **4** with $(DHQD)_2PYDZ$[15] in 1.5 : 1 *tert*-BuOH–H_2O proceeded slowly because of the limited solubility of the sterol, but gave 24(*R*),25-dihydroxycholesterol (**5**) in 82% yield and with 96 : 4 diastereoselectivity. Treatment of **5** with methanesulfonyl chloride (4 equiv) and pyridine (20 equiv) in CH_2Cl_2 at 23 °C effected conversion to the 3,24-

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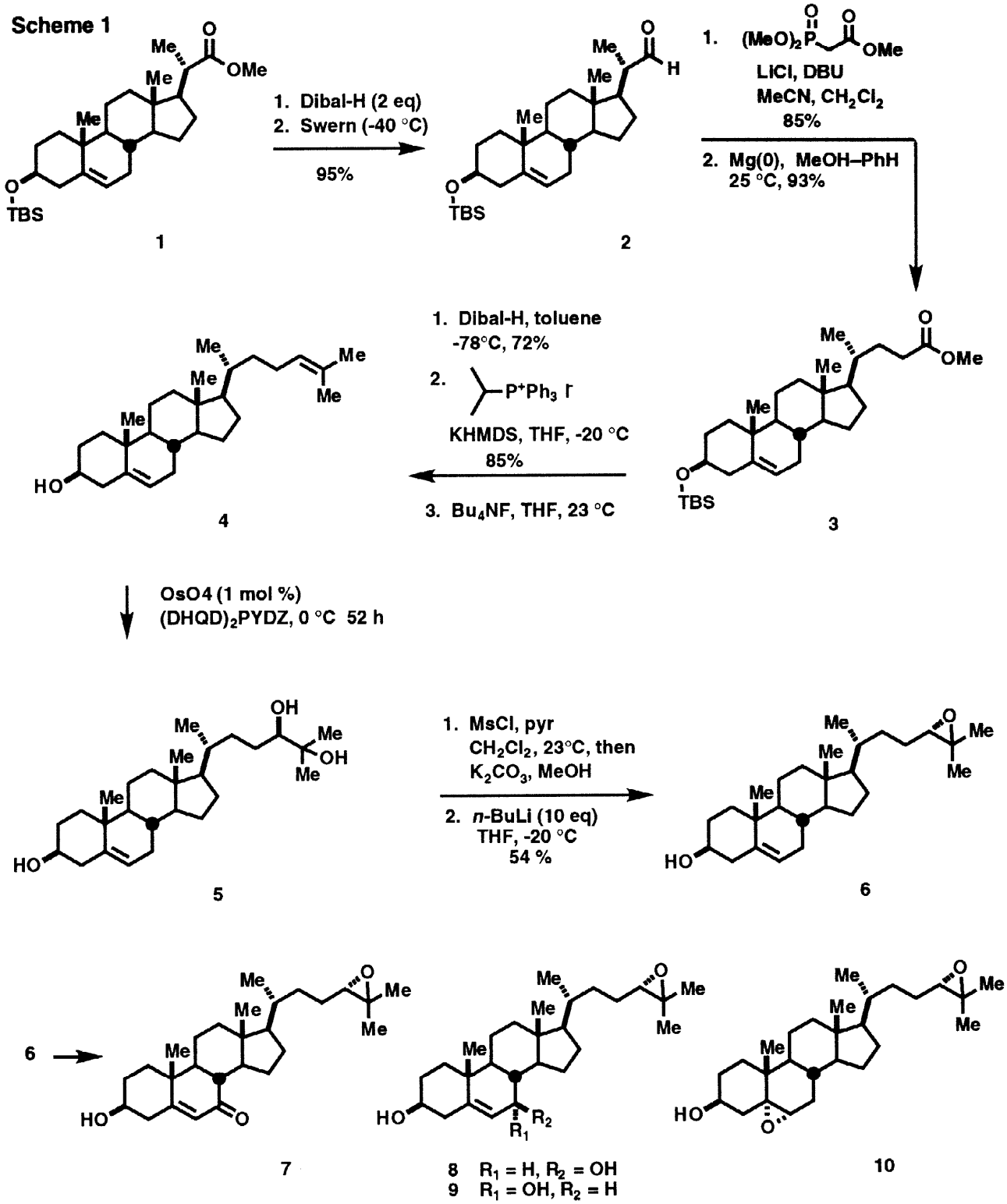
bismesylate of **5** which upon exposure to K_2CO_3 in MeOH gave the 24,25-epoxide. Removal of the 3-mesylate with excess *n*-BuLi in THF at -20 °C affords 24(*S*),25-epoxycholesterol **6**. Asymmetric dihydroxylation of **4** with OsO_4 and $(DHQ)_2PHAL$[16] produced in good yield with high diastereoselectivity the 24(*S*)-triol, which was similarly converted to 24(*R*),25-ECH.

In order to test the effect of B-ring oxygenation on oxysterol binding to and activation of LXR we have synthesized a number of further oxidation products of **6** including the 7-keto derivative **7**, the 7β- and 7α-hydroxy derivatives **8** and **9** and the 5,6-α-epoxide **10**. The 7-ketone **7** was produced from **6** by the sequence: (1) reaction with benzoyl chloride, triethylamine and 4-*N*,*N*-dimethylaminopyridine in CH_2Cl_2 at 23 °C to form the 3-benzoate (97%), (2) oxidation with 1.1 equiv of *tert*-BuOOH and a catalytic amount of cupric pivalate in C_6H_6 at 70 °C for 12 h to give the 7-ketone (50%)[17] and (3) benzoate cleavage by use of 1% NaOH in CH_3OH–THF (5 : 1) at 23 °C for 1.5 h to afford **7** (87%). The 7β-alcohol **8** was prepared from the benzoate of **7** by reduction with $NaBH_4$–$CeCl_3$ in THF–CH_3OH (2 : 1) at 23 °C for 10 min[18] and benzoate cleavage with NaOH in CH_3OH–THF as described for **7**. The 7α-alcohol **9** was similarly prepared from the benzoate of **7** by use of L-Selectride in THF at -78 °C for 5 h[18] and subsequent benzoate cleavage. Epoxidation of the 3-acetate of **6** with *m*-chloroperoxybenzoic acid at -40 °C in CH_2Cl_2 and acetate cleavage with K_2CO_3–CH_3OH at 23 °C provided predominantly the 5,6-α-epoxide **10** (5,6-α/5,6-β selectivity 8 : 1).

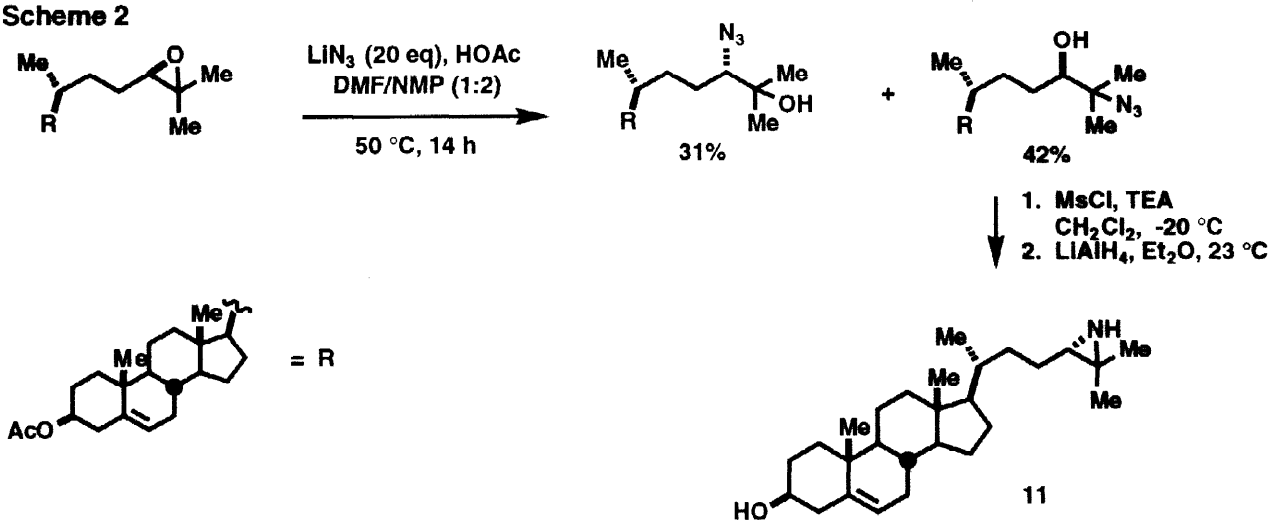
The 24(*S*),25-imino analog of epoxide **6** 24(*S*),25-iminocholesterol (**11**) was prepared from 24(*R*),25-epoxycholesterol acetate as shown in Scheme 2. The imino analog **11** was of special interest to test the effect of replacing the epoxide oxygen in **6** by the more strongly basic imino function on binding and activation of LXR.

Biological evaluation of the various synthetic ligands described herein is reported in detail elsewhere,[11] but can be summarized briefly as follows. The most active compounds with respect to binding to LXRα and whole cell functional activation of LXRs were 24(*S*),25-EC (**6**), 7α-hydroxy-24(*S*),25-EC (**9**) and 5,6-α-24(*S*),25-diEC (**10**). Interestingly, while **6** and **9** also activated LXRβ, **10** did not. Thus, **10** is the first known selective activator of LXRα vs LXRβ. Poor binding to and activation of LXRα and LXRβ were found for compounds **5**, **7**, **8** and **11**. The imino sterol **11** was also found to be toxic to cells. Based on the hypothesis that a H-bond accepting oxygen function at C(24) can lead to good binding to and activation of LXRs, we tested the 3β-alcohol of methyl ester **3** and also the corresponding *N*,*N*-dimethylamide. As predicted, both were active. Remarkably, the dimethylamide showed 80% of the functional activity of 24(*S*),25-EC vs LXRα and 110% vs LXRβ. Thus, 3β-hydroxycholenic acid dimethylamide appears to be an interesting lead compound for treatment of hypercholesteremia.[19]

Scheme 1


1. Dibal-H (2 eq)
2. Swern (-40 °C)
95%
1. (MeO)2P(O)CH2C(O)OMe
LiCl, DBU
MeCN, CH2Cl2
85%
2. Mg(0), MeOH–PhH
25 °C, 93%
1. Dibal-H, toluene
-78°C, 72%
2. P+Ph3 I-
KHMDS, THF, -20 °C
85%
3. Bu4NF, THF, 23 °C
OsO4 (1 mol %)
(DHQD)2PYDZ, 0 °C 52 h
1. MsCl, pyr
CH2Cl2, 23°C, then
K2CO3, MeOH
2. n-BuLi (10 eq)
THF, -20 °C
54 %
6
8 R1 = H, R2 = OH
9 R1 = OH, R2 = H

Scheme 2



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