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A concise synthesis of β-sitosterol and other phytosterols

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ABSTRACT

A convenient synthesis of sidechain-modified phytosterols is achieved via a temporary masking of the stigmasterol 5,6-alkene as an epoxide. Following performance of the desired modification, the alkene is regenerated through a mild deoxygenation. The approach is applied to the syntheses of β-sitosterol and campesterol acetate, and suggests a facile route to the (*Z*)-isomers of Δ^{22-23} phytosterols.

1. Introduction

Phytosterols and their derivatives are widely applied in the food and cosmetic industries, and have recently received a great deal of attention as nutraceutical additives [1–3]. Phytosterols have also attracted attention as inhibitors of sarcoplasmic reticulum calcium ATPase and potassium ion channels [4,5]. As part of a collaboration investigating the structural influences on uptake and processing of sterol esters [6], we required semipreparative amounts of β-sitosterol. However, β-sitosterol is commercially available in preparative amounts only as mixtures with other phytosterols, including stigmasterol, campesterol, and/or brassicasterol (Fig. 1); reported separations are relatively laborious [7,8].

Two routes have been reported for the synthesis of β-sitosterol from stigmasterol, which is available in pure form. The first, selective hydrogenation of the sidechain Δ^{22-23} alkene [9], was found to produce β-sitosterol contaminated with varying amounts of recovered stigmasterol as well as the fully saturated stigmastanol [10]. The second approach, which has been applied to the synthesis of sitosterol and related sterols (Fig. 2), circumvents the need for selective hydrogenation by protecting the Δ^{5-6} alkene as a cyclopropyl carbinyl ether [11,12]. Following hydrogenation of the Δ^{22-23} double bond, solvolysis of the cyclopropane reintroduces both the C_3-alcohol and the Δ^{5-6} alkene. Although we found the latter approach very useful as a means of obtaining very pure samples of β-sitosterol, semipreparative applications were challenging in terms of removal of sterol methyl ether byproducts.

We now report a new strategy for the synthesis of sidechain-modified phytosterols based upon protection of the Δ^{5-6} alkene as an epoxide. The approach is illustrated with syntheses of β-sitosterol and campesterol acetate.

2. Experimental

2.1. General experimental procedures

AlI_3 and $Cu(MnO_4)_2$ were prepared by literature procedures [13,14]. All other reagents and solvents were used as supplied commercially, except CH_2Cl_2 (CaH_2) and THF (Na, Ph_2CO) which were distilled from the indicated reagent under an atmosphere of N_2. Melting points are uncorrected. Unless noted, NMR spectra were acquired at 400 MHz (^1H) or 100 MHz (^{13}C) in $CDCl_3$; individual peaks are reported as: multiplicity, integration, coupling constant in Hz. IR spectra were recorded as neat films on a ZnSe crystal with selected absorbances reported in cm^{-1}. Mass spectroscopy was conducted at the Nebraska Center for Mass Spectrometry.

2.2. Stigmasterol acetate (**2**)

Stigmasterol acetate was prepared as a white solid (97%, mp 138–140 °C) by a variant of the procedure of Wang et al. [15]. Other physical and spectral data were identical to literature values.

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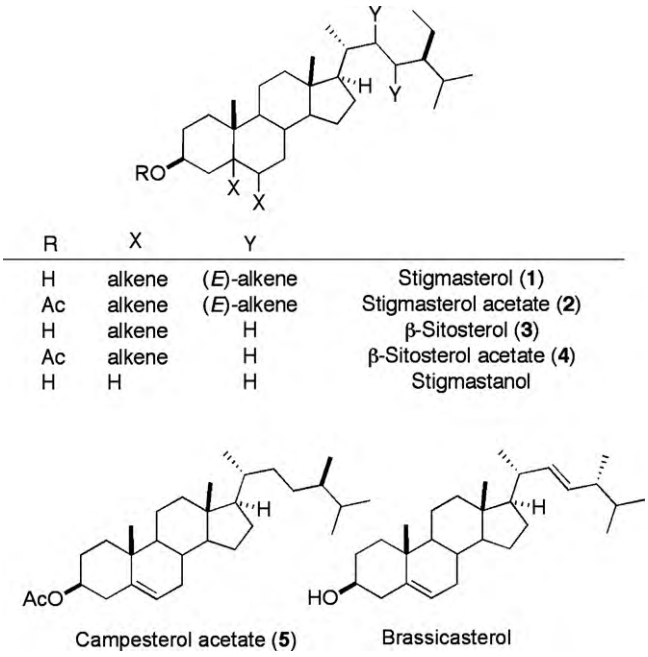


Fig. 1. Structural relationship of phytosterols.

2.3. 5α,6α- and 5β,6β-Epoxides of stigmasterol acetate (**6a**, **6b**)

A mixture of $KMnO_4$ (10 g, 60 mmol) and $CuSO_4{\cdot}5H_2O$ (5.0 g, 20 mmol) was finely ground in a mortar and pestle [14]. Water (0.5 mL) was added, and the slightly wet mixture was transferred to the reaction flask. To the stirred suspension of this mixture in 25 mL CH_2Cl_2 was added stigmasterol acetate (**2**, 2.12 g, 4.51 mmol), followed by *t*-BuOH (2.5 mL). The reaction was heated to reflux for 1 h and cooled to room temperature. The reaction mixture was filtered through a silica pad, which was washed with ether. The residue obtained after concentration was recrystallized from CH_3OH to give a white solid (1.59 g, 75%) with mp 125–126 °C. NMR data indicated the product was a 1:6 mixture of the α- (**6a**) and β- (**6b**) epoxides of stigmasterol acetate [14]. Repeating this reaction with 2.23 g of stigmasterol acetate afforded 1.80 g (78%) of a 1:6 mixture of **6a** and **6b**.

Approach to 5α,6α- and 5β,6β-epoxides of stigmasterol acetate (**6a**, **6b**) via peracid epoxidation: to a 0 °C solution of **2** (0.308 g, 0.66 mmol) in CH_2Cl_2 (20 mL) was added mCPBA (0.170 g, 0.76 mmol). After 4 h at 0 °C, the reaction was diluted with sat. aq. K_2CO_3 (80 mL) and the aqueous layer was extracted with CH_2Cl_2 (3× 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography (hexane/EtOAc, 90:10) to afford 0.257 g (83%) of a white solid which was a 2.6:1 mixture of α- (**6a**) and β-isomers (**6b**) according to ^1H NMR.

2.4. 5α,6α- and 5β,6β-Epoxy sitosterol acetate (**7a**, **7b**)

To a solution of the 1:6 mixture of **6a** and **6b** (1.35 g, 3.0 mmol) in EtOAc (150 mL) was added 10% Pd/C (0.32 g), and the stirred reaction mixture was placed under an atmosphere of H_2 (balloon) for 12 h. The reaction mixture was filtered through a Celite pad, and the filtrate evaporated to furnish white solid (1.28 g, 96%, mp 113–114 °C) as a 1:6 mixture of epoxides **7a** and **7b** [8]. Repeating this reaction on 1.76 g of **6a**/**6b** afforded 1.75 g (99%) of a 1:6 mixture of **7a** and **7b**.

2.5. β-Sitosterol acetate (**4**)

The 1:6 mixture of epoxides **7a** and **7b** (470 mg, 1.0 mmol) was dissolved in 2:1 CH_3CN/CH_2Cl_2 (30 mL). Aluminum triiodide was added (610 mg, 1.5 mmol) and the resulting mixture was stirred at room temperature for 10 min. The reaction was quenched with aq. 10% $Na_2S_2O_3$ (100 mL) and the resulting mixture was extracted with CH_2Cl_2 (3× 100 mL). The combined organic layers were dried over Na_2SO_4, and the residue from the concentrated filtrate was purified by flash chromatography (hexane/EtOAc, 95:5) to give 360 mg (80%) of **4** as a white solid: mp 111–112 °C, $[\alpha]_D = -34.5$ ($CHCl_3$, $c = 1.0$). Other physical data were identical to values reported in the literature [11]. Repeating this reaction on 1.70 g of **7a**/**7b** afforded 1.40 g (85%) of **4**.

2.6. β-Sitosterol (**3**)

To a solution of β-sitosterol acetate (**4**, 240 mg, 0.47 mmol) in 1:1 CH_3OH:CH_2Cl_2 (30 mL) was added K_2CO_3 (140 mg, 1.01 mmol). The reaction mixture was stirred at room temperature for 12 h and then concentrated under vacuum. The residue was extracted with 30 mL CH_2Cl_2. The organic layer was washed with 30 mL water and dried over Na_2SO_4. The filtered organic layer was concentrated and the residue was purified through flash chromatography (hexane/EtOAc, 80:20) to give 220 mg (93%) of β-sitosterol **3** as a white solid. Mp 134–135 °C, $[\alpha]_D = -37.0$ ($CHCl_3$, $c = 1.0$). Elemental analysis calculated for $C_{29}H_{50}O$: C 83.60, H 11.96; found: 83.99, 12.15. Other spectral properties were identical to values reported in the literature [11]. Repeating this reaction on 1.35 g of **4** afforded 1.20 g (98%) of beta sitosterol (**3**).

2.7. (*S*)-2,3-Dimethylbutan-1-ol (**8**)

(*S*)-2,3-Dimethylbutan-1-ol **8** was prepared as a colorless liquid (overall yield 60%, $[\alpha]_D = 4.4$, $CHCl_3$, $c = 1.0$) by the procedure of Tietze, affording a product with spectral data identical to literature values [16].

2.8. (*S*)-2-(2,3-Dimethylbutylthio)benzothiazole (**9**)

To a mixture of dimethylbutanol **8** (102 mg, 1.00 mmol), 2-mercaptobenzothiazole (183 mg, 1.10 mmol) and PPh_3 (288 mg, 1.10 mmol) in freshly distilled THF (4 mL) was added diisopropyl

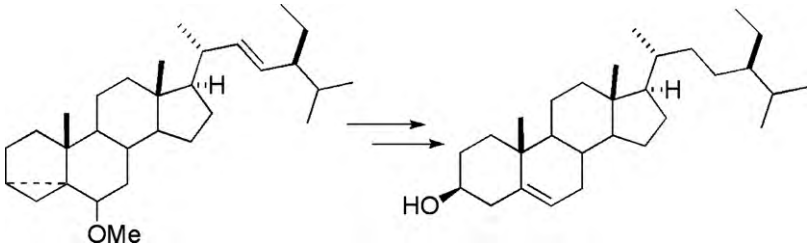


Fig. 2. Selective saturation of cyclopropyl carbinyl ether.

azodicarboxylate (DIAD, 0.21 mL, 1.10 mmol) dropwise at 0 °C under argon. The reaction was stirred for 3 h at 0 °C and then quenched with water. The aqueous layer was extracted with EtOAc (3× 10 mL) and the combined organic layers were dried over anhydrous Na_2SO_4. The filtered organic layer was concentrated in vacuo and the residue purified by flash chromatography (hexane/EtOAc, 99:1) to afford thioether **9** (228 mg, 91%) as a light yellow oil. $[\alpha]_D = 42.3$ ($CHCl_3$, $c = 1.6$); IR 2957, 1455, 1426, 1057, 991, 752 cm^{-1}; ^1H NMR: δ 7.89 (d, $J = 8.1$, 1H), 7.75 (d, $J = 8.1$, 1H), 7.42 (t, $J = 7.2$, 1H), 7.29 (t, $J = 7.2$, 1H), 3.50 (dd, $J = 12.7, 4.8$, 1H), 3.18 (dd, $J = 12.7, 8.2$, 1H), 1.88–1.77 (m, 2H), 1.04 (d, $J = 6.7$, 3H), 0.99 (d, $J = 6.6$, 3H), 0.94 (d, $J = 6.6$, 3H); ^{13}C NMR: δ 167.75, 153.38, 135.15, 125.99, 124.08, 121.44, 120.91, 38.87, 38.78, 31.62, 20.37, 17.96, 15.29; HRFAB-MS (m/z) $[M-H]^+$ calcd for $[C_{13}H_{18}NS_2]^+$: 252.0881, found: 252.0875.

2.9. (S)-2-(2,3-Dimethylbutylsulfonyl)benzothiazole (**10**)

A 0 °C solution of **9** (183 mg, 0.73 mmol) in EtOH (10 mL) was oxidized with ammonium heptamolybdate tetrahydrate (1.8 g, 1.46 mmol) and 30% H_2O_2 (2.5 mL, 21.9 mmol) for 2 h. The mixture was extracted with EtOAc (3× 10 mL) and the combined organic extracts were washed with brine (3× 10 mL). The dried organic layers were filtered and the residue obtained upon concentration was purified by flash chromatography (hexane/EtOAc, 90:10) to afford sulfone **10** (177 mg, 86%) as a pale yellow oil. $[\alpha]_D = 15.5$ ($CHCl_3$, $c = 3.4$); IR 2961, 1470, 1324, 1140, 1085, 758 cm^{-1}; ^1H NMR: δ 8.23 (d, $J = 7.9$, 1H), 8.03 (d, $J = 7.9$, 1H), 7.68–7.59 (m, 2H), 3.59 (dd, $J = 14.4, 3.5$, 1H), 3.31 (dd, $J = 14.1, 8.9$, 1H), 2.29–2.19 (m, 1H), 1.82–1.73 (m, 1H), 1.10 (d, $J = 6.9$, 3H), 0.89 (d, $J = 6.8$, 3H), 0.85 (d, $J = 6.9$, 3H); ^{13}C NMR: δ 166.66, 152.70, 136.74, 128.00, 127.67, 125.43, 122.38, 58.83, 38.68, 32.47, 19.23, 17.89, 15.93; HRFAB-MS (m/z) $[M-H]^+$ calcd for $[C_{13}H_{18}NO_2S_2]^+$: 284.0779, found: 284.0778.

2.10. (3β,5α,6α)- and (3β,5β,6β)-Pregnane-20α-carboxaldehyde-5,6-epoxy-3-yl acetate (**11a**, **11b**)

A −78 °C solution of **6a**, **6b** (~1:6 mixture, 100 mg, 0.21 mmol) in 10 mL of 50/50 CH_2Cl_2/MeOH was treated with a gaseous stream of ozone (2% O_3/O_2) for 5 min. The solution was purged with pure oxygen and then solvent was removed under vacuum. The residue was redissolved in 10 mL of 10/90 H_2O/AcOH and treated with zinc powder (55 mg, 0.84 mmol). The reaction mixture was stirred for 2 h at room temperature and then extracted with 50 mL CH_2Cl_2. The organic layer was washed with water (3× 25 mL), then dried over anhydrous Na_2SO_4. The filtered organic layer was concentrated and the residue purified by flash chromatography (hexane/EtOAc, 90:10) to afford a 1:6 mixture of epoxides **11a** and **11b** as a white solid (81 mg, 99%), mp 87–8 °C. IR: 2950, 1727, 1367, 1262, 1238, 1042, 783 cm^{-1}; ^1H NMR: δ 9.57 (d, $J = 3.3$, 0.76H, β), 9.55 (d, $J = 3.3$, 0.16H, α), 4.99–4.91 (m, 0.14H, α), 4.81–4.73 (m, 0.87H, β), 3.09 (d, $J = 2.2$, 0.88H, β), 2.90 (d, $J = 4.2$, 0.13H, α), 2.38–2.31 (m, 1H), 2.13–1.82 (m, 9H), 1.54–0.89 (m, 20H), 0.7 (s, 3H); ^{13}C NMR: δ 204.95, 170.52, 71.25, 63.41, 62.48, 55.39, 51.05, 50.93, 49.42, 42.89, 39.43, 37.95, 36.67, 35.06, 32.41, 29.74, 27.17, 26.97, 24.54, 21.84, 21.30, 17.03, 13.40, 12.11; HRFAB-MS (m/z) $[M-Li]^+$ calcd for $[C_{24}H_{36}LiO_4]^+$: 395.2774, found: 395.2778.

2.11. (3β,5α,6α,22Z)- and (3β,5β,6β,22Z)-Ergost-5,6-epoxy-22-en-3-yl acetate (**12a**, **12b**)

To a 78 °C solution of sulfone **10** (62 mg, 0.22 mmol) in THF (5 mL) was dropwise added LiHMDS (0.22 mL, nominally 1 M in THF, 0.22 mmol). The reaction was stirred for 1 h, whereupon the mixture of aldehydes **11a** and **11b** (~1:6, 85 mg, 0.22 mmol) was added in 5 mL of THF. Stirring was continued for 1 h, and reaction was gradually warmed to room temperature. The reaction was quenched by 15 mL water and extracted with EtOAc (3× 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The residue obtained upon concentration was purified by flash chromatography (hexane/EtOAc, 95:5) to afford a 1:16 mixture of epoxides **12a** and **12b** as a white solid (90 mg, 90%), mp 145–147 °C. IR: 2950, 2867, 1743, 1368, 1037, 764 cm^{-1}; ^1H NMR: δ 5.02 (dd, $J = 10.9, 9.9$, 2H), 4.83–4.73 (m, 1H), 3.09 (d, $J = 2.0$, 0.95H, β), 2.91 (d, $J = 4.4, 0.06$, α), 2.43–2.33 (m, 1H), 2.21–1.80 (m, 9H), 1.68–0.83 (m, 31H), 0.69 (s, 3H); ^{13}C NMR: δ 170.56, 135.13, 131.22, 71.34, 63.58, 62.52, 56.27, 56.02, 51.02, 42.18, 39.72, 38.32, 38.01, 36.68, 35.04, 34.48, 33.35, 32.43, 29.74, 28.32, 27.20, 24.15, 21.92, 21.34, 20.55, 20.36, 19.94, 18.63, 17.06, 12.06; HRFAB-MS (m/z) $[M-H]^+$ calcd for $[C_{30}H_{49}O_3]^+$: 457.3682, found: 457.3668.

2.12. 5α,6α- and 5β,6β-Epoxides of campesterol acetate (**13a**, **13b**)

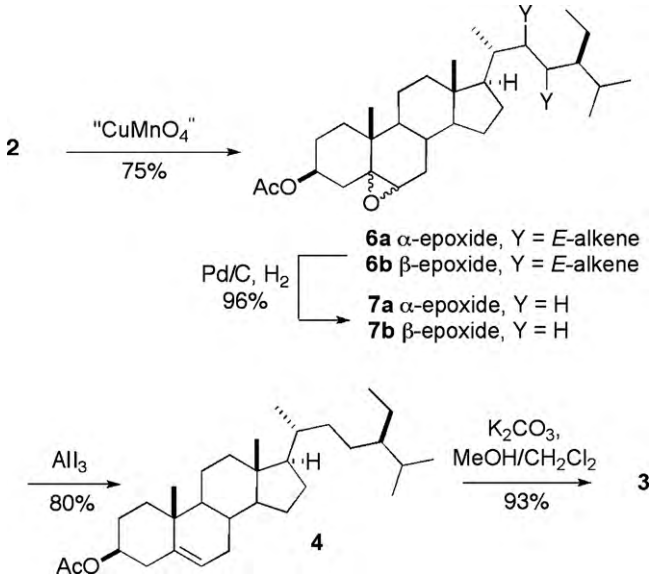
The mixture of epoxides **12a** and **12b** (30 mg, 0.065 mmol) was dissolved in 5 mL EtOAc. 10% Pd/C (7 mg) was added, and the reaction mixture was stirred at room temperature under an atmosphere of H_2 (balloon) for 12 h. The reaction mixture was filtered through a Celite pad, and the filtrate evaporated to a white solid (28 mg, 94%, mp 110–111 °C) as 1:9 mixture of epoxides **13a** and **13b**. IR: 2953, 2867, 1729, 1367, 1263, 1043, 784 cm^{-1}; ^1H NMR: δ 5.01–4.93 (m, 0.15H, α), 4.83–4.73 (m, 0.96H, β), 3.09 (d, $J = 2.1$, 0.9H, β), 2.90 (d, $J = 4.4$, 0.1H, α), 2.12–1.8 (m, 8H), 1.58–0.77 (m, 37H), 0.65 (s, 3H); ^{13}C NMR: δ 170.54, 71.34, 63.58, 62.51, 56.19, 56.14, 50.97, 42.28, 39.78, 38.81, 38.01, 36.66, 35.82, 35.03, 33.65, 32.47, 32.41, 30.26, 29.73, 28.14, 27.21, 24.18, 21.92, 21.31, 20.19, 18.66, 18.24, 17.03, 15.37, 11.76; HRFAB-MS (m/z) $[M-H]^+$ calcd for $[C_{30}H_{51}O_3]^+$: 459.3838, found: 459.3820.

2.13. Campesterol acetate (**5**)

The mixture of epoxides **13a** and **13b** (28 mg, 0.061 mmol) was dissolved in 2:1 CH_3CN/CH_2Cl_2 (3 mL). Aluminum triiodide (37 mg, 0.091 mmol) was added and the resulting mixture was stirred at room temperature for 40 min. The reaction was quenched with aq. 10% $Na_2S_2O_3$ (10 mL) and the resulting mixture was extracted with CH_2Cl_2 (3× 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the residue from the concentrated filtrate was purified by flash chromatography (hexane/EtOAc, 95:5) to give 24 mg (91%) campesterol acetate (**5**) as a white solid. Mp 130–131 °C, $[\alpha]_D = -32$ ($CHCl_3$, $c = 0.7$); IR 2954, 1730, 1367, 1247, 1037, 735 cm^{-1}; ^1H NMR: δ 5.39 (d, $J = 4.8$, 1H), 4.66–4.58 (m, 1H), 2.33 (d, $J = 7.9$, 2H), 2.05 (s, 3H), 1.90–1.84 (m, 2H), 1.59–0.78 (m, 38H), 0.68 (s, 3H); ^{13}C NMR: δ 170.56, 139.66, 122.66, 73.99, 56.69, 56.08, 50.02, 42.31, 39.73, 38.84, 38.12, 36.99, 36.59, 35.90, 33.70, 32.43, 31.90, 31.86, 30.27, 28.24, 27.78, 24.29, 21.46, 21.03, 20.22, 19.32, 18.70, 18.26, 15.38, 11.87; HRFAB-MS (m/z) $[M-Na]^+$ calcd for $[C_{30}H_{50}O_2Na]^+$: 465.3709, found: 465.3703. Elemental analysis calculated for $C_{30}H_{50}O_2$: C 81.20, H 11.38; found: 81.39, 11.39. The ^1H NMR data matched that of a literature report [17].

3. Results and discussion

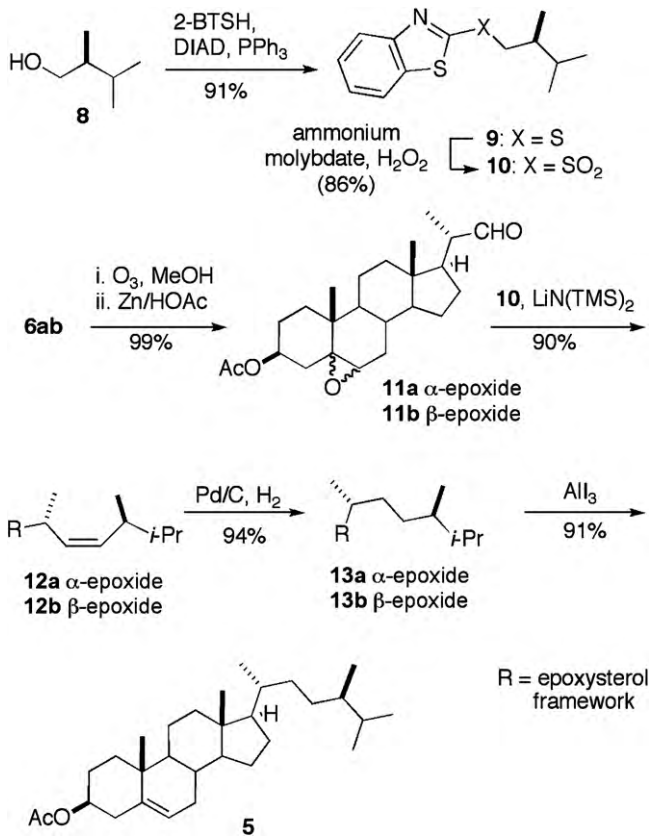
Our synthesis of β-sitosterol (**3**) is illustrated in Scheme 1. Selective epoxidation of the Δ^{5-6} alkene of stigmasterol acetate (**2**) with copper permanangate formed a 6:1 mixture of the 5β,6β- and 5α,6α-epoxides **6b** and **6a** [14,18]. Hydrogenation over Pd/C cleanly furnished a 1:6 mixture of sitosterol epoxides **7a** and **7b**. Deoxygenation of the saturated epoxides with AlI_3 [13] proceeded



Scheme 1. Synthesis of sitosterol.

rapidly to furnish a good yield of β-sitosteryl acetate **4**. Saponification afforded pure β-sitosterol (**3**), with mp 134–135 °C and $[\alpha]_D = -37.0$ [8,19].

Epoxidation with the commercially available peracid mCPBA also gave good selectivity for the Δ^{5-6} alkene, but now produced a 2.6:1 mixture of stigmasterol oxides favoring the α-isomer (**6a**). Hydrogenation proceeded uneventfully to furnish the corresponding mixture of sitosterol oxides **7a** and **7b**. However, attempted deoxygenation under the same conditions as employed



Scheme 2. Synthesis of campesterol acetate.

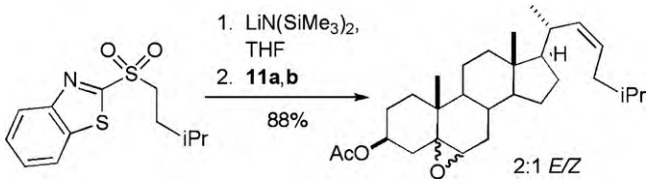


Fig. 3. Generality of *Z*-selective olefination.

earlier (AlI_3, 10 min, CH_3CN/CH_2Cl_2) now furnished only 33% of β-sitosterol acetate (**4**), accompanied by a significant amount (estimated >60% by mass) of a more polar product which yellowed immediately upon exposure to room light. The formation of the byproduct could be avoided almost completely by allowing the deoxygenation to proceed for 40 min. Alternatively, the byproduct could be converted to **4** by treatment with additional AlI_3. The results suggest that the deoxygenation of the α- and β-epoxides proceeds at very different rates, with the 5α,6α diastereomer (**6a**) reacting via the intermediacy of a semistable iodohydrin.

As illustrated in Scheme 2, our strategy also provides a facile means of preparing other sidechain-modified phytosterols. For example, ozonolysis of the mixture of **6a**/**6b** furnished an approximately 1:6 mixture of aldehydes **11a** and **11b**. Julia-Kocienski olefination, using an enantiomerically pure sulfone (**10**) derived from (*S*)-2,3-dimethylbutanol (**8**) [16] furnished exclusively alkene **12** [20], corresponding to the monoepoxide of the *Z*-isomer of crinosterol [21]. Hydrogenation, followed by deoxygenation of the epoxide as before, furnished campesterol acetate (**5**).

The selective formation of *Z*-alkenes is unusual in Julia couplings [22] and we investigated the olefination of **11a** and **11b** with a known sulfone derived from isobutyl alcohol (Fig. 3) [23]. This reaction also selectively furnished the *Z*-alkene; the lower selectivity (2:1) compared with that observed for the synthesis of **12a** and **12b** may reflect the reduced degree of steric encumbrance in this model system. The ability to readily prepare *Z*-isomers of phytosterols opens the door to a number of steroid analogs not available from synthetic routes based upon Claisen rearrangements of C_{22} allylic alcohols [12].

4. Conclusions

The formation of β-sitosterol has been achieved in 52% overall yield from commercially available stigmasterol using relatively simple chemistry and via easily purified intermediates. The core strategy, protection of the Δ^{5-6} alkene as an epoxide, holds potential for the synthesis of other phytosterols as well as unnatural analogs.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data for this article, consisting of ^1H and ^{13}C NMR spectra for compounds **3**–**13**, can be found in the online version, at doi:10.1016/j.steroids.2010.05.016.

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