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Phytosterols: a Healthy Alternative to Cholesterol?

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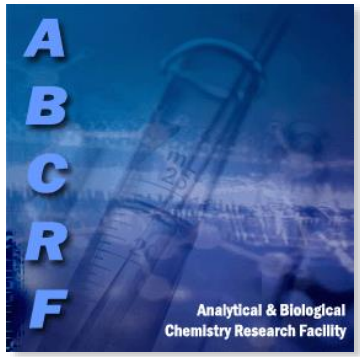

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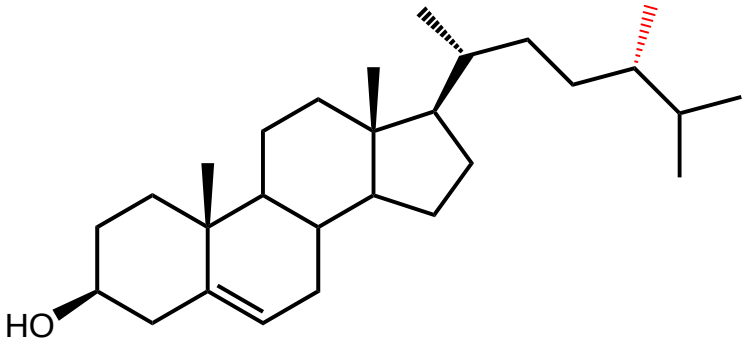
Phytosterols: a Healthy Alternative to Cholesterol?

Graphical Abstract



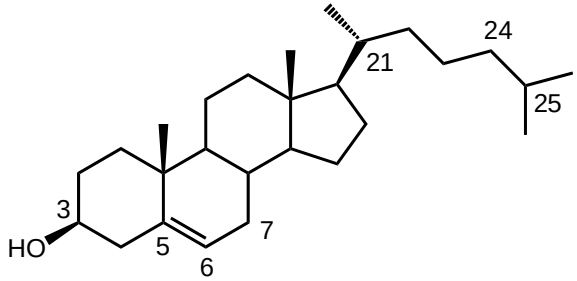
Abstract: Phytosterols are increasingly used as health supplements in functional foods and are associated with having both positive and negative effects on health.[1] In contrast to the heavily promoted health benefits of dietary phytosterol supplementation, a number of groups have identified adverse health effects of phytosterols: induction of endothelial dysfunction and increased size of ischaemic stroke; inhibition of cell growth; aggressive vascular disease in sitosterolaemic patients.[2,3] Given this disparity, an investigation of their full individual biological profile is imperative in order to assure food safety.

Herein we describe the de novo synthesis of pure phytosterols in multigram scale and report the first synthesis of the key phytosterol Dihydrobrassicasterol and its oxides along with a comparison of routes to Campesterol.[4,5] A detailed spectroscopic analysis is included with full assignment of the 13C NMR of both phytosterols, mixtures and their precursors leading to the potential use of NMR as a tool for analysis of these sterol mixtures.

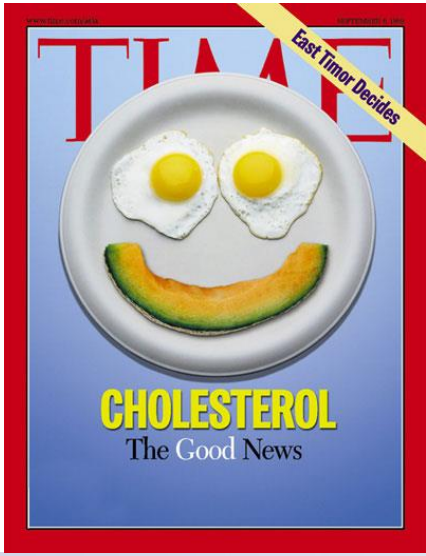
A comprehensive toxicological profile of these key phytosterol oxide products (POPs) identifies critical problems with the use of phytosterol mixtures as food additives.[5,6,7]

Keywords: Cholesterol; Phytosterol Oxidation Products; β-Sitosterol; Campesterol; DIhydrobrassicasterol

Introduction



- Cholesterol
- Numberous roles within the body
  - Many specific biological processes designed for the transport of cholesterol from one bodily compartment to another
  - Roles in membrane stability and a ligand in many essential functions
  - Biogenic precursor of many compounds involved in growth
- Associated with poor health
  - Cholesterol testing; Low cholesterol diets; Cholesterol replacement
- Considerable health budget costs
  - Drive to reduce impact of high cholesterol levels to life
- Cardiovascular disease; Atherosclerosis/atherogenesis; Stroke; Transient Ischemic Attack; Poor life expectancy
- Many of these adverse effects can be attributed to the presence of Cholesterol Oxidation Products (COPs)
  - Oxides can be generated enzymatically *in vivo* or chemically on storage/preparation



Phytosterols

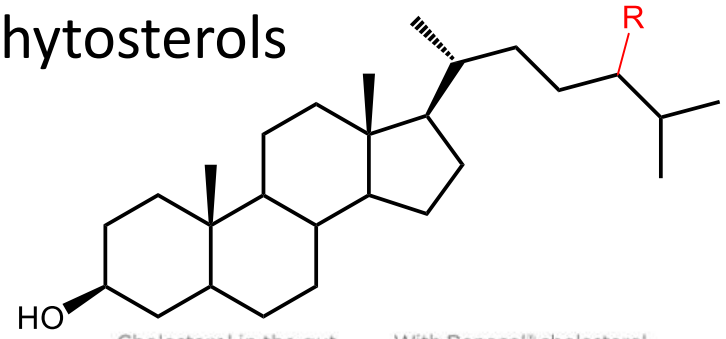
- Phytosterols are plant derived sterols that differ in structure to cholesterol by substitution at C-24
- Phytosterols are proven to give beneficial cholesterol lowering effects when supplemented in the food supply.
  - Mixed micelle formation resulting in decreased absorption
  - Complex mechanisms results in the absorption of about 50% of cholesterol, but <5% of plant sterols and <0.5% of plant stanols

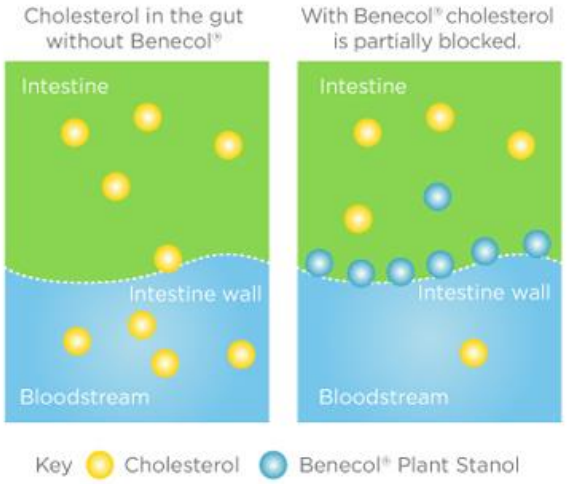
- Phytosterol esters are currently incorporated into many functional foods such as spreads, yoghurt, milk, salad dressing, soy, cheese, fruit drinks, sausages and breads
  - Projected intakes could be as high as 13 g/day.

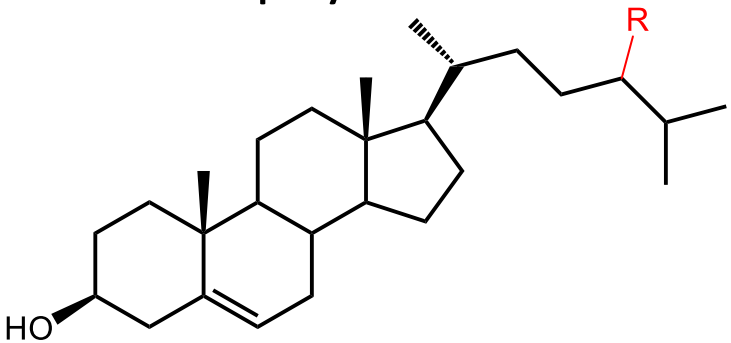
- Examples include Benecol phytostanol esters & Flora Pro-active phytosterol esters
- This has significant consequences for their oxidative susceptibility

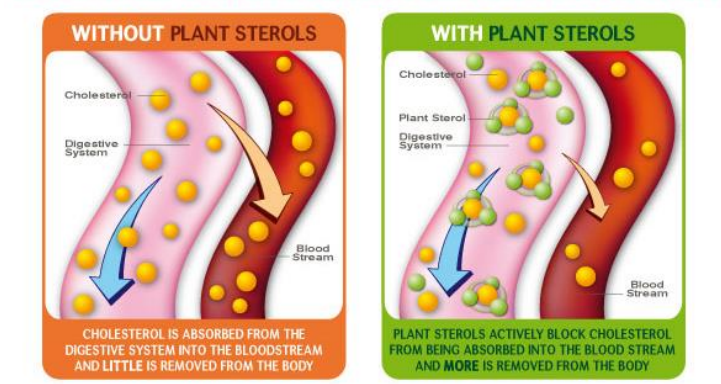
Phytosterol/Phytostanol

- Operate on same principle of mixed micelle formation resulting in reduced intestinal absorption of Cholesterol and hence lower physiological levels
- Significant chemical difference between the phytostanols and phytosterols



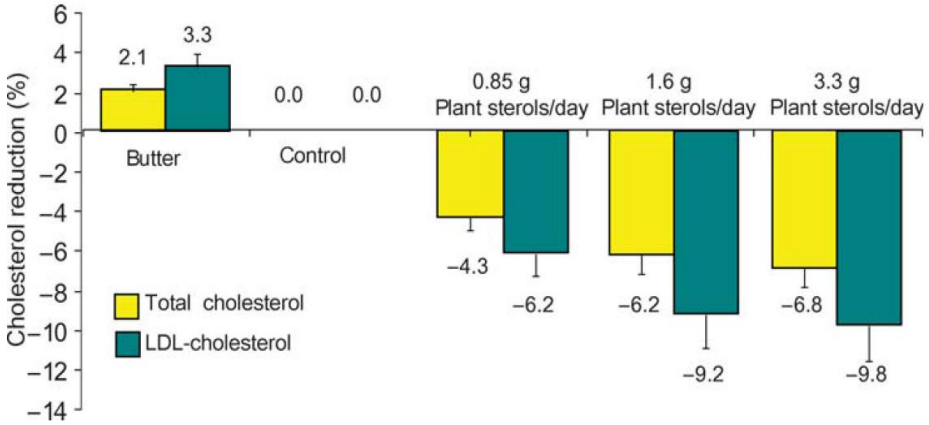


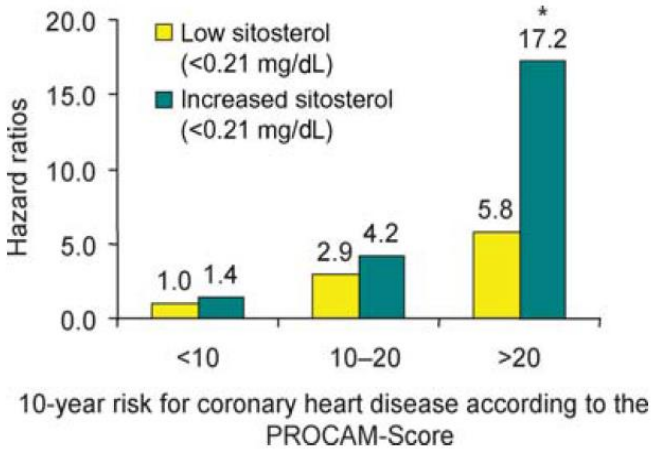




Phytosterol Controversy

- Obvious short term cholesterol lowering benefits
- Questionable long term benefits





- No data available indicating that functional foods supplemented with plant sterol esters reduce cardiovascular events.
- For patients with the hereditary disease of sitosterolaemia, data from epidemiological studies, as well as recently published *in vitro* and *in vivo* data suggest that plant sterols potentially induce negative cardiovascular effects.

Weingartner et al. (2009). *Eur Heart J,* 91, 101-106.

Why the Controversy?

- Phytosterols are natural compounds
- Levels required for Cholesterol lowering effect are difficult to attain by diet alone
  - Normal Western-type diet contains about 200–500 mg cholesterol, 200–400 mg plant sterols, and about 50 mg of plant stanols

Difficult to attain by diet

2. Katan MB, Grundy SM, Jones P, et al. Mayo Clin Proc 2003; 78:965-78

- The consequence of increasing this component artificially is that impurities/metabolites/oxidation products will now be ingested (or formed) at much higher levels and could have serious ramifications

Emergence of Phytosterol Oxides

- Phytosterols are added to fortified foods as a phytosterol blend for economic reasons containing (but not limited to):
  - β-Sitosterol, Stigmasterol, Campesterol and Dihydrobrassicasterol
  - Ratio is dependent on source (commonly Palm oil)

- Proven health benefits via the lowering of low density lipoprotein cholesterol concentrations
  - Close structural similarity to cholesterol
  - Potential problems due to their oxidative susceptibility.
  - Oxidation to form hydroxy, epoxy, keto and triol derivatives

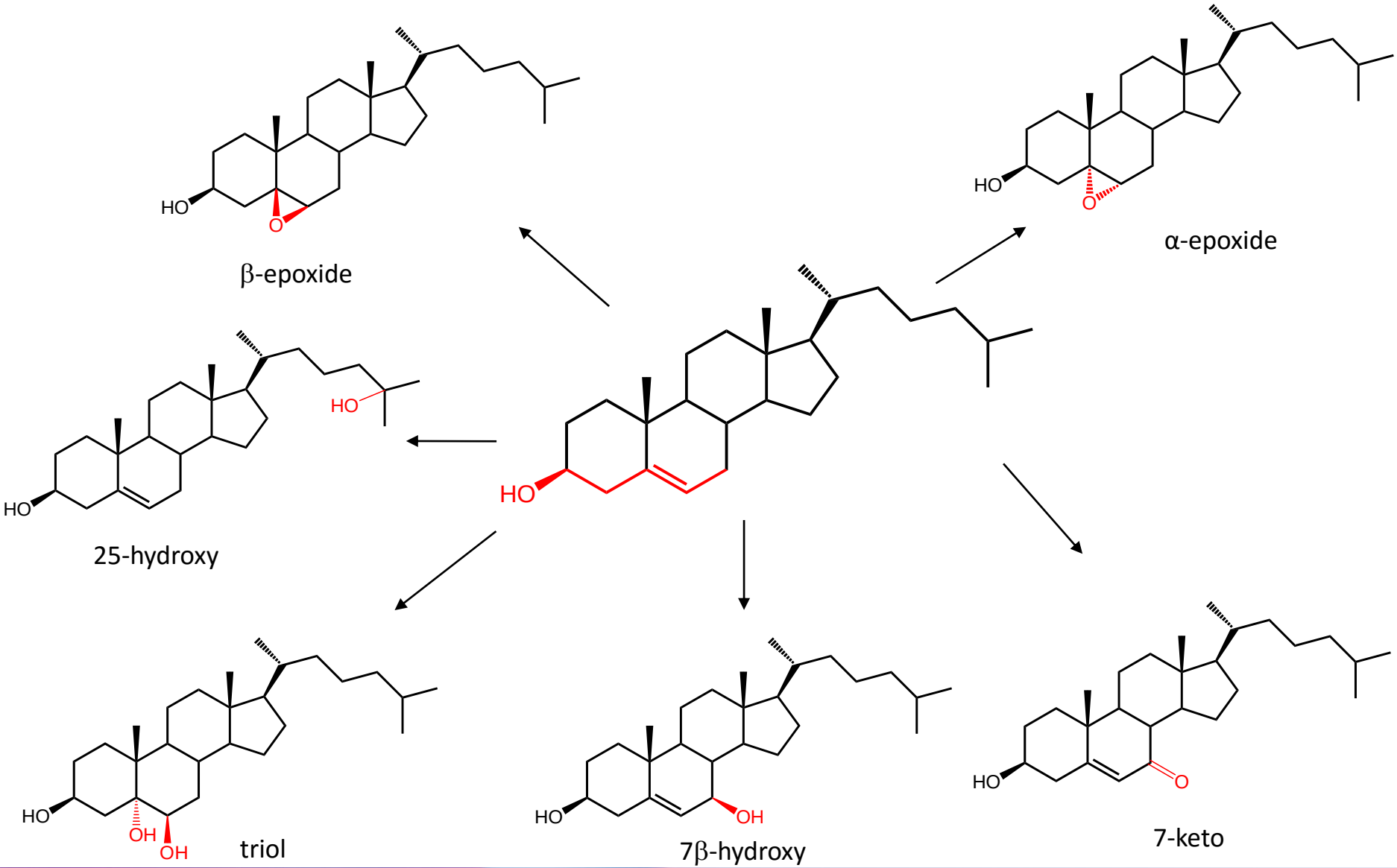
- Collectively known as Phytosterol Oxidation Products (POPs).
- These derivatives have diverse biological functions of eminent interest to clinicians

Oxysterols

- Cholesterol Oxidation Products (COPs) and Phytosterol Oxidation products (POPs)
- Significant metabolic and environmental derivatives of sterols
- Associated with toxicity, cellular adhesion and the initiation and progression of major chronic diseases including atherosclerosis, neurodegenerative processes, diabetes, kidney failure, and ethanol intoxication.
- COPs are most widely studied due to their prevalence *in vivo*
- POPs less well known and specific limitations in the availability of standards
  - Targets for novel synthesis and biological evaluation
  - Food safety and security
  - Variance of natural sources and now prevalence of phytosterol-enriched foods risks oxyphytosterols as dietary/metabolic lipid components.
  - Food industry Ireland/EU one of the biggest markets

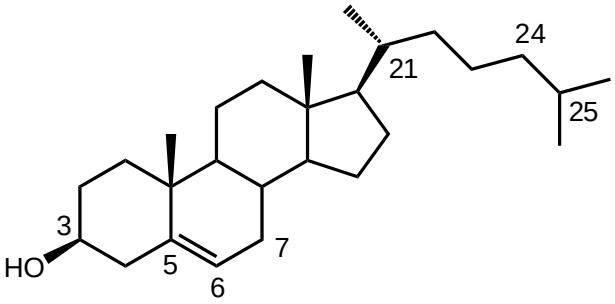
O'Callaghan. et al. (2014) *Biochemical Biophysical Research Communications, 446 (3):786-791*

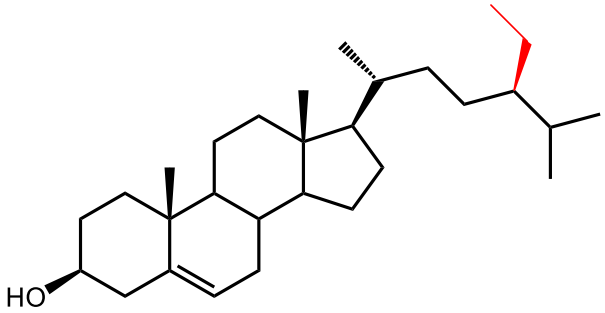
Cholesterol and its oxides

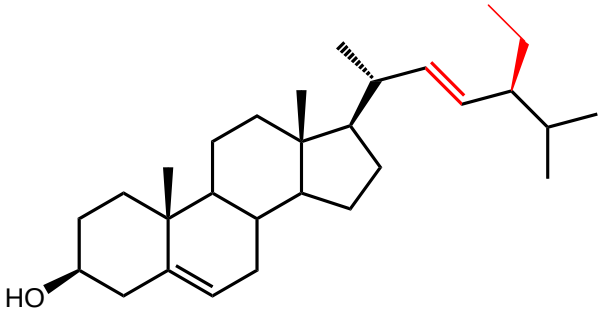


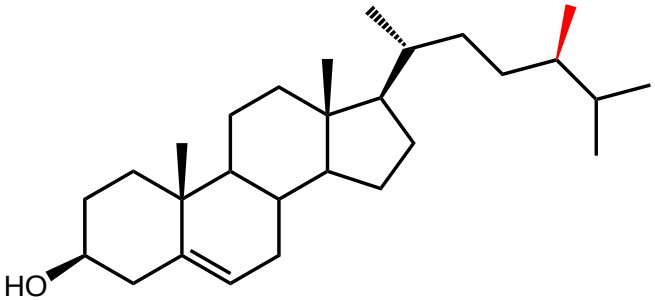
Outline

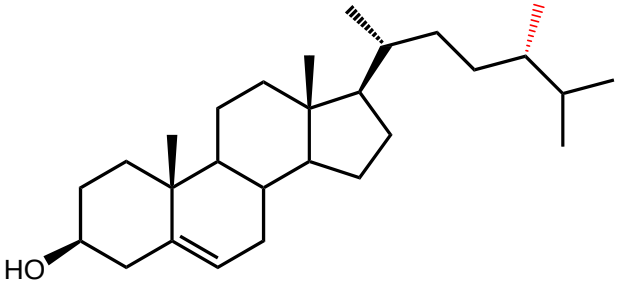
- Identify the possible toxic oxidation products of phytosterols.
- Ensure consumer protection by safeguarding against their production in the food supply.
- Compare: Cholesterol, β-Sitosterol, Stigmasterol, Campesterol and Dihydrobrassicasterol



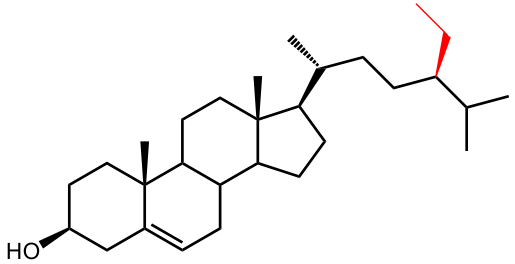








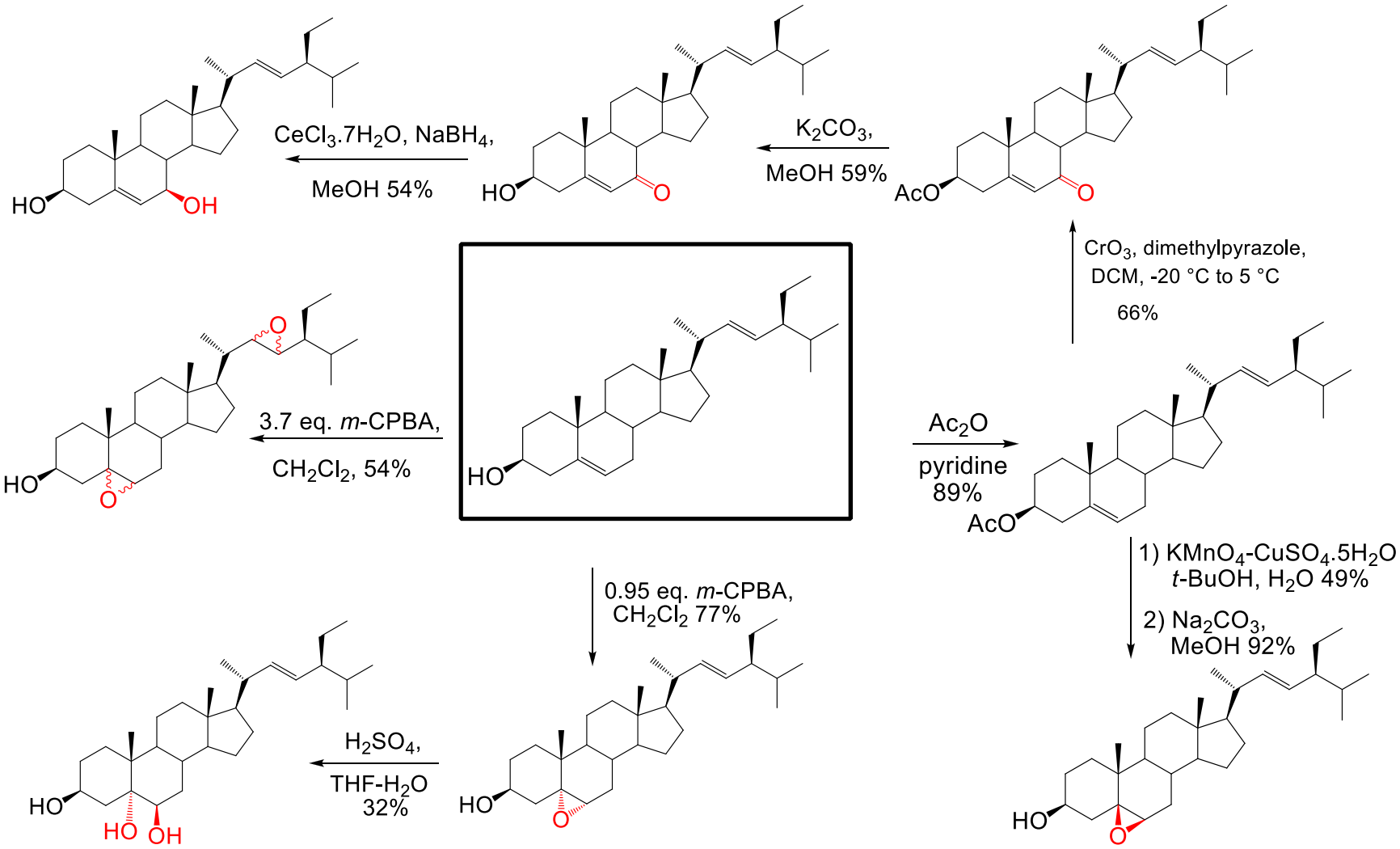
Background – Phytosterol Oxides



- Research has shown that phytosterol blends do indeed undergo oxidation on food processing and preparation
  - forming 7-hydroxysterols, 7-ketosterols, 5,6-epoxysterols, triols and 25-hydroxysterols.
- Research in this area will assist in designing dietary strategies to minimise the impact of phytosterol oxides in our food supply
- Research on potential toxicity of phytosterol oxides is considerably hindered by the lack of pure compounds as reference standards
- Cost: Campesterol very expensive commercially (for 65% purity)
- Dihydrobrassicasterol – not available
- Initial work on phytosterol oxides published the toxicity profile of 5 oxides of β-sitosterol as proof of concept.
- Consequently have set out to identify, synthesise and purify an extensive range of phytosterol oxides as pure compounds and assess their biological activity and toxicity.
- These standards can be used in the assessment of phytosterol mixtures incorporated into functional foods ensuring consumer protection.

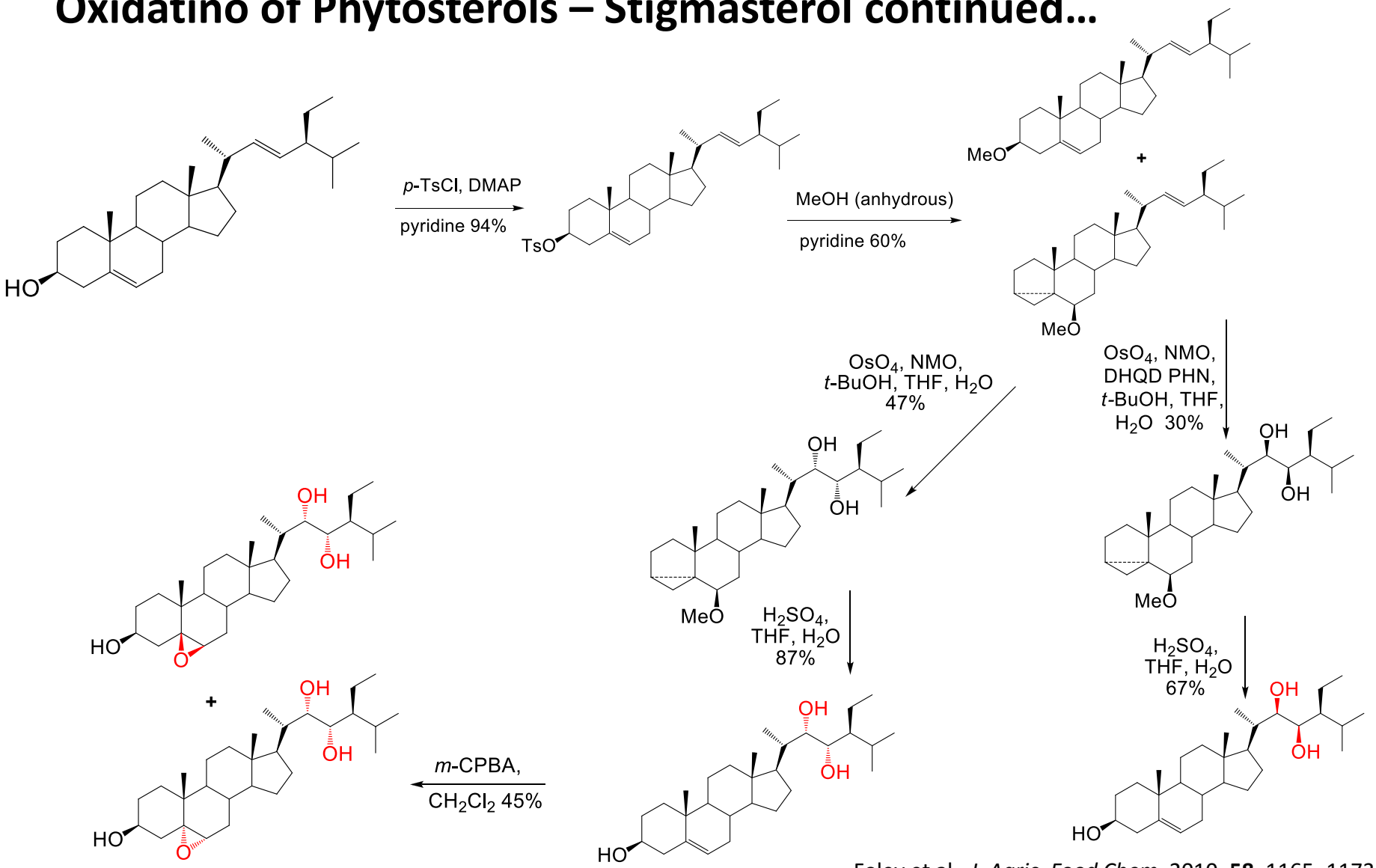
McCarthy F, Ryan E, O'Brien NM, Maguire AR et al. (2005). *Organic and Biomolecular Chemistry* 3, 3059-3065.
Ryan E, Chopra J, McCarthy F, Maguire AR, O'Brien NM (2005). *British Journal of Nutrition* 94, 443-451.
Maguire LS, Konoplyannikov M, Ford A, Maguire AR, O'Brien NM (2003). *British Journal of Nutrition* 90, 767-775.
Lampi A-M et al. (2002) *J. Chromatogr. B.* 777, 83-92.
Bortolomeazzi R. et al. (2003). *J. Agric. Food Chem.*, 51, 375-382.
Soupas L et al. (2004). *J. Agric. Food Chem.*, 52, 6485-6491
Grandgirard A. et al. (2004). *British Journal of Nutrition* 91, 101-106.

Oxidation of Phytosterols - Stigmasterol



Foley et al. *J. Agric. Food Chem.* 2010, **58**, 1165–1173

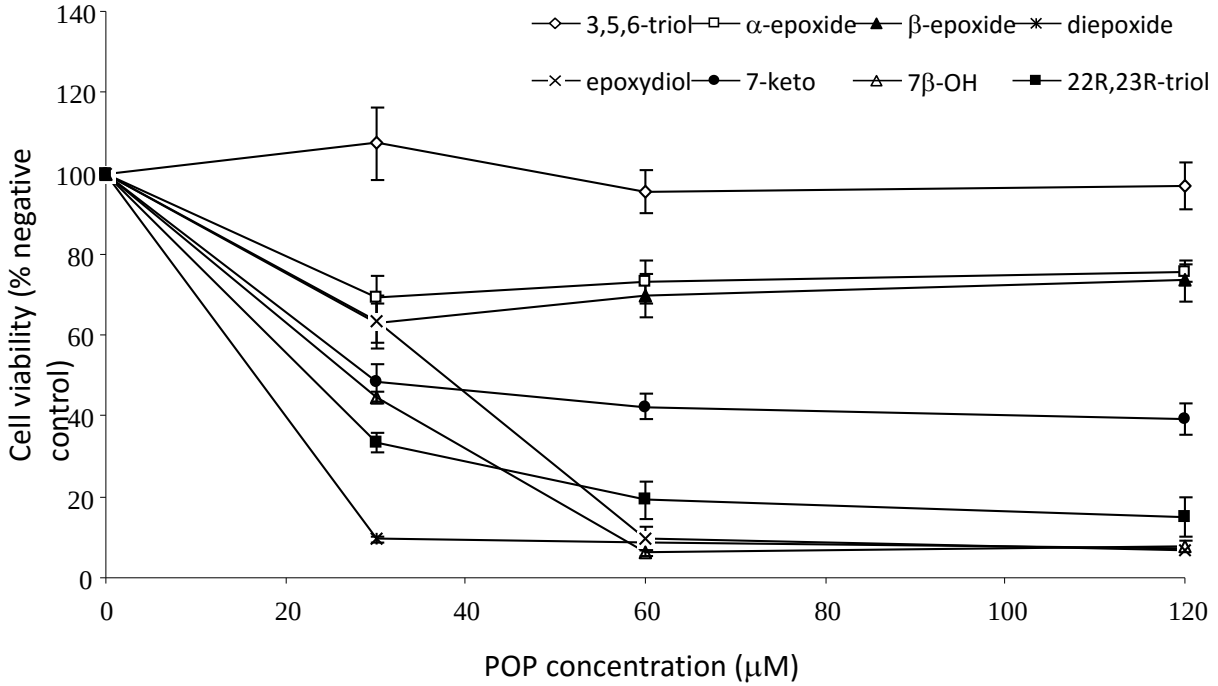
Oxidatino of Phytosterols – Stigmasterol continued…



Foley et al. *J. Agric. Food Chem.* 2010, **58**, 1165–1173

Viability of U937 cells exposed to Stigmasterol oxide for 24 hr as measured by the MTT assay

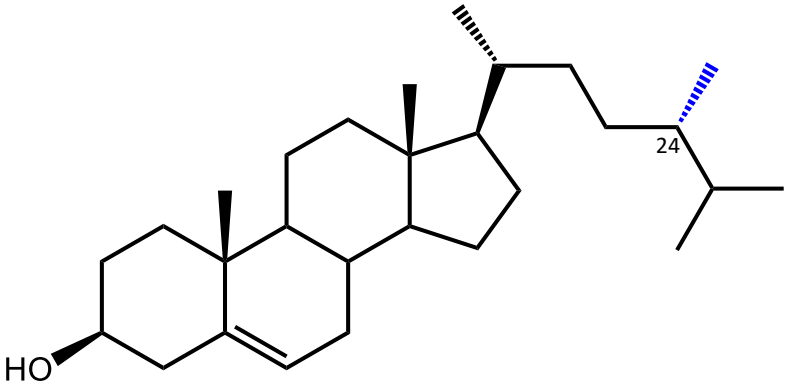
- All oxides assessed for cytotoxicity and apoptotic effects
- Cell viability: FDA/EtBr assay, MTT assay
- Apoptosis: Hoechst 33342 staining, DNA fragmentation, Caspase-3 activity, Bcl-2 content, Cellular glutathione content



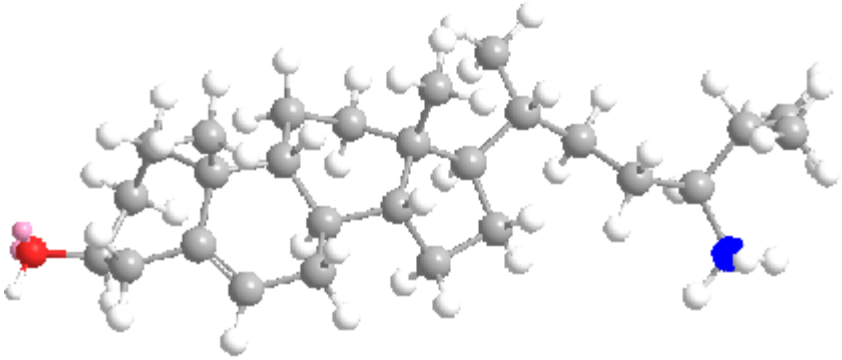
- The sidechain oxide derivatives of stigmasterol were found to be the most cytotoxic of all the derivatives tested in the U937 cell line
- At 30μM diepoxide, the mode of cell death was almost exclusively apoptotic
- The pathway of apoptosis involved glutathione depletion, caspase-3 activation and Bcl-2 down-regulation
- Further investigation warranted

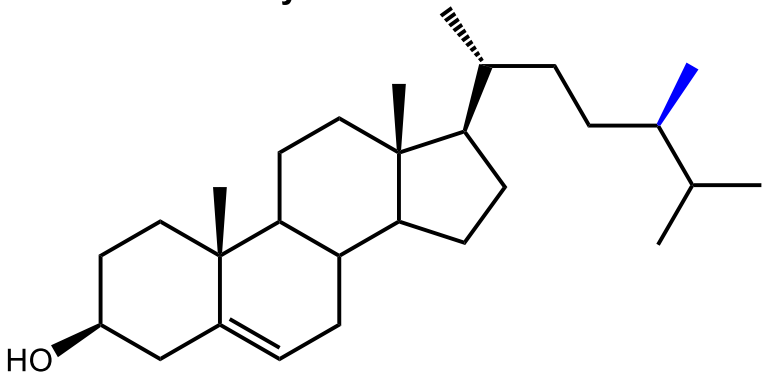
O'Callaghan et al. *J. Agric. Food Chem.* 2010, **58**, 10793–10798

Dihydrobrassicasterol and Campesterol

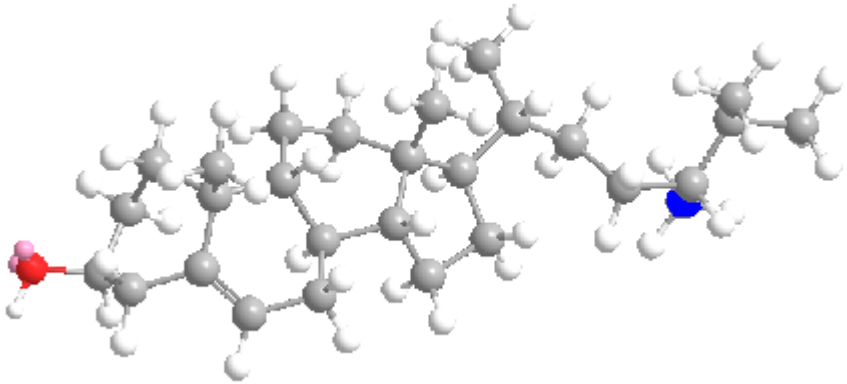


Dihydrobrassicasterol





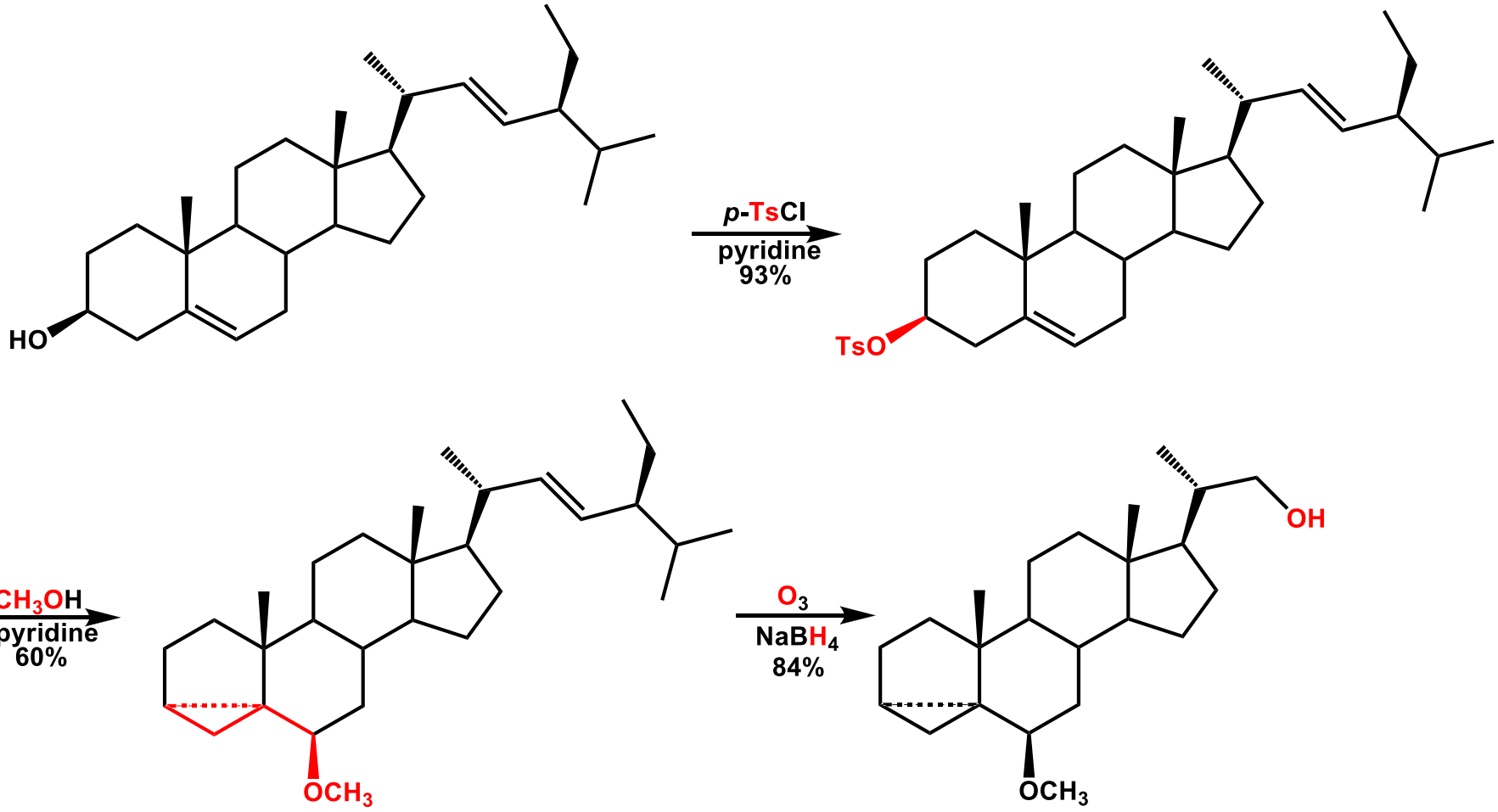
Campesterol



- Differ at C-24, where the methyl group is *S* stereochemistry in Dihydrobrassicasterol and *R* in Campesterol.

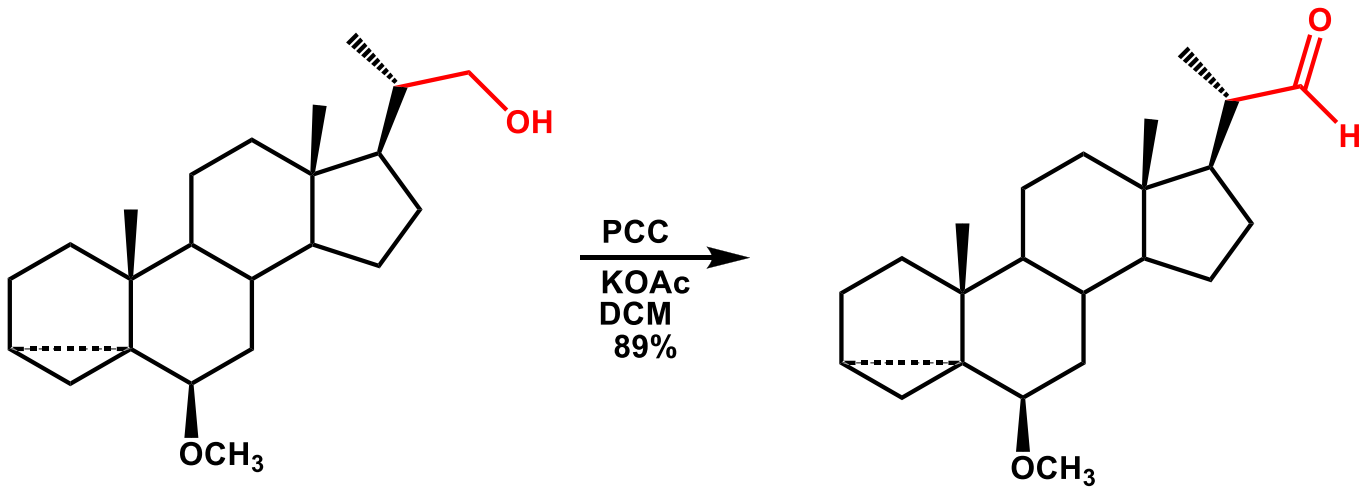
- The synthesis of the precursor side chain therefore is different for each diastereomer.

Synthesis of Steroid Buiding Block



McCarthy, F. O.; O'Brien, N. M.; Ryan, E.; Maguire, A. R.; *Org. Biomol. Chem*. **2005**, 3, 3059-3065

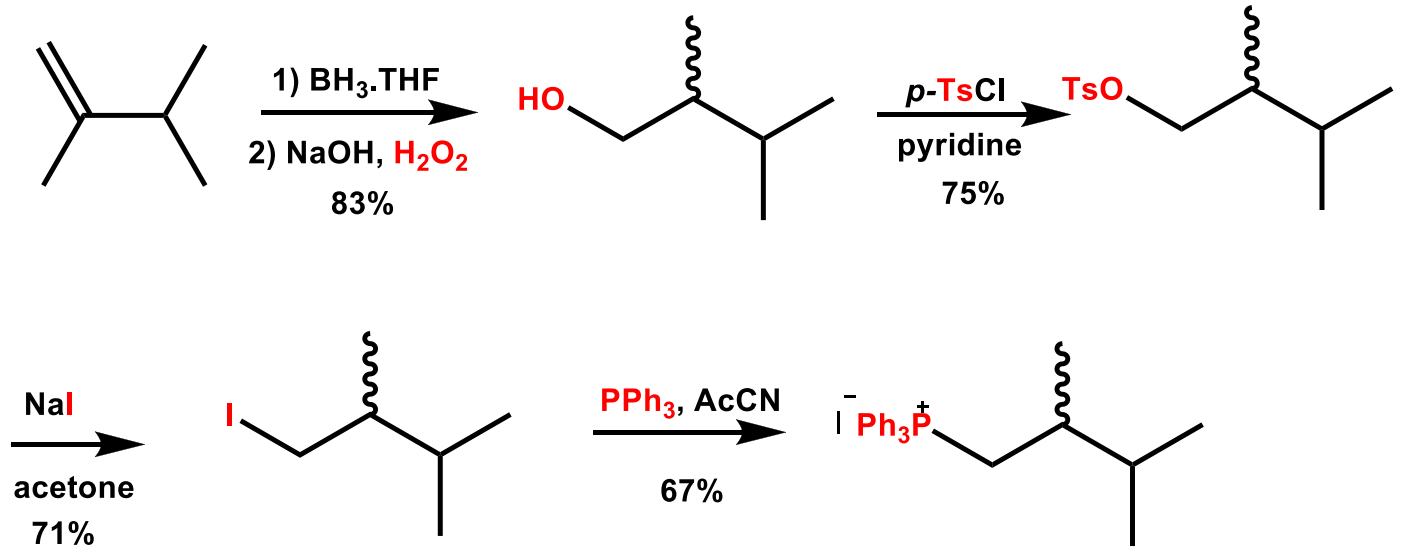
Oxidation step



Oxidation method	% yield	Challenges encountered
O_3, PPh_3	30 - 79	Variable yields
Swern oxidation	88	Sulfur present impeded next step
PCC oxidation	89	none

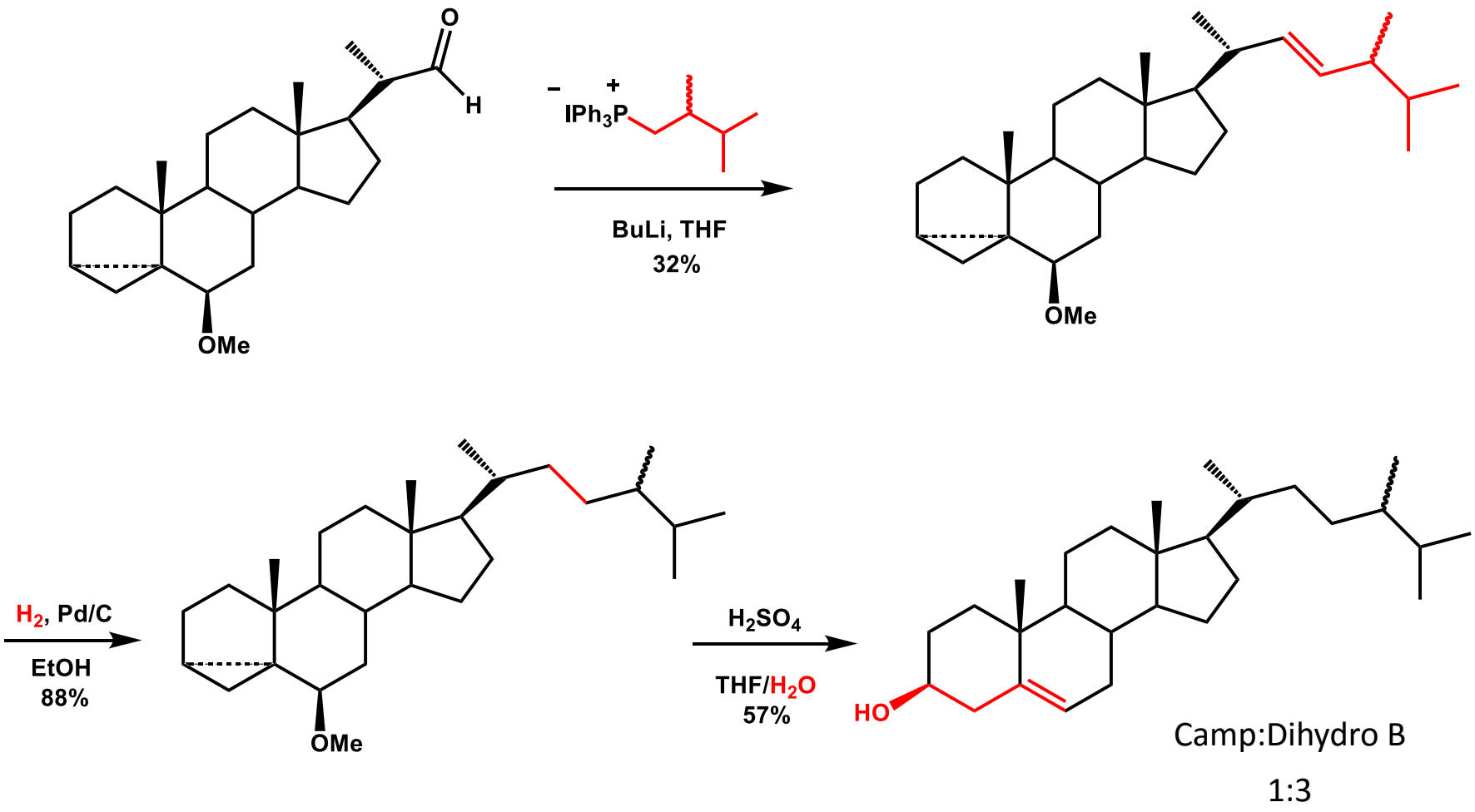
McCarthy, F. O.; O'Brien, N. M.; Ryan, E.; Maguire, A. R.; *Org. Biomol. Chem*. **2005**, 3, 3059-3065

Test synthesis of Wittig racemic salt



- Wittig salt is directly synthesized from a commercially available alkene
- Steps proceed with little difficulty and are high yielding.
- Lack of stereocontrol in hydroboration limits this approach

Test synthesis of Racemic mixture

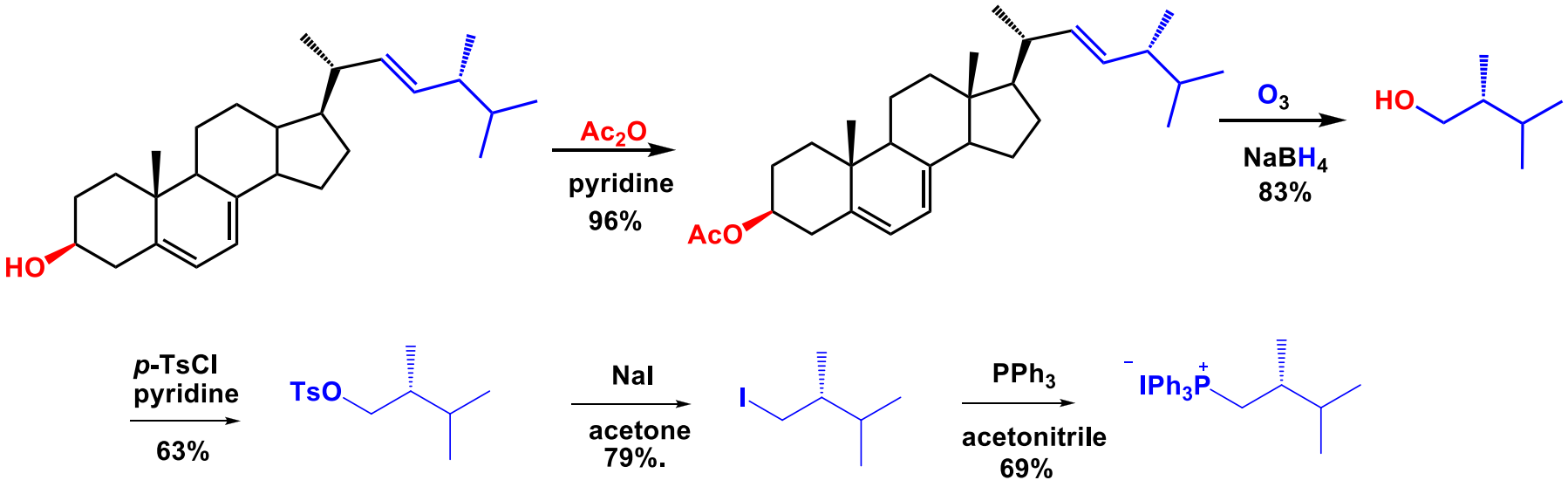


Camp:Dihydro B

1:3

Dihydrobrassicasteol side chain

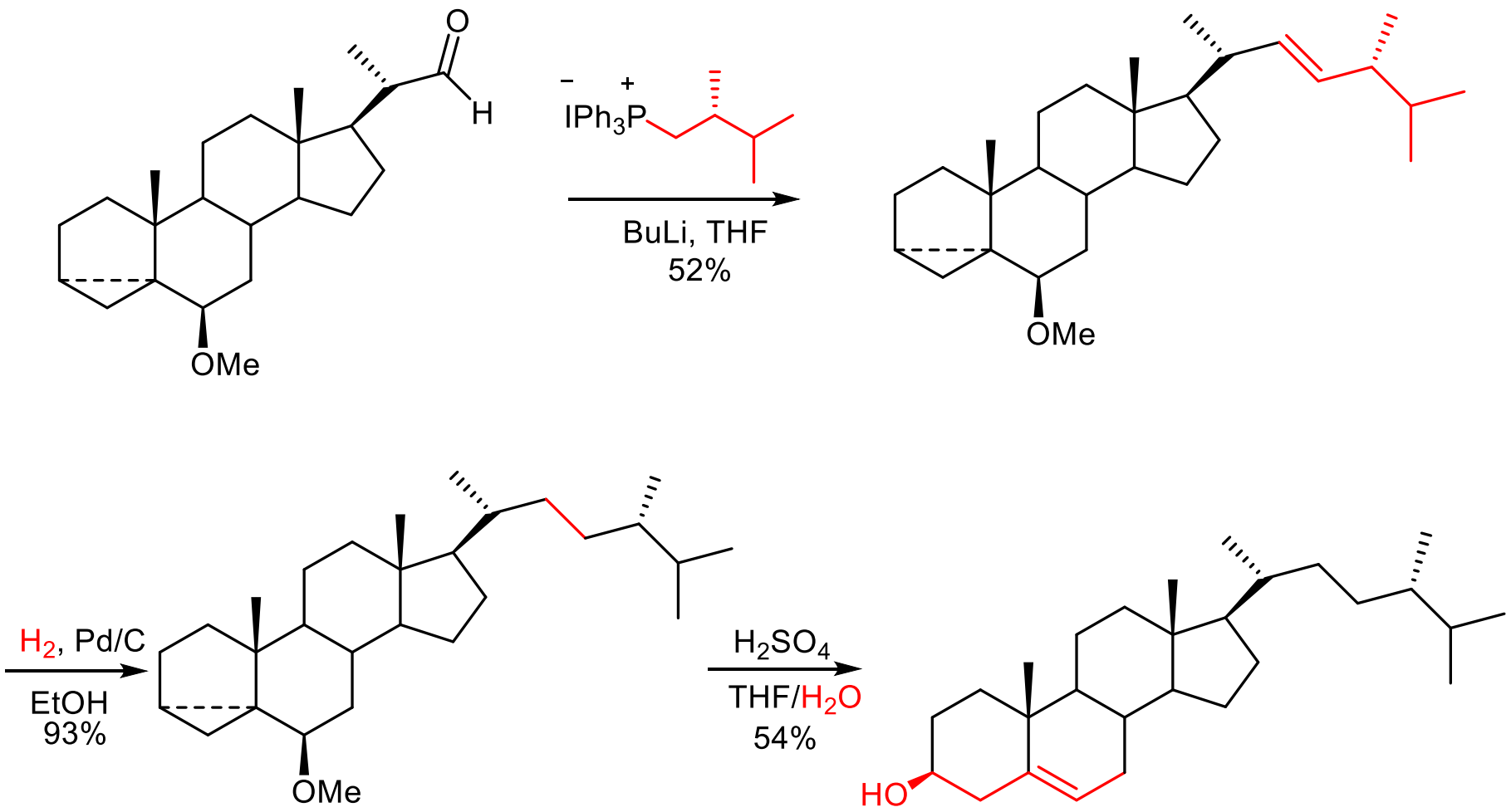
- Needed a novel approach to the key side chain
- In Ergosterol nature provides the lead



- Ergosterol is converted to alcohol in good yield by ozonolysis and reduction.
- This synthetic pathway is two steps using inexpensive starting materials.
- Stereochemical integrity is maintained.

Hua, Z. H. et al. *J. Org. Chem.* 2005, *70*, 9849-9856.

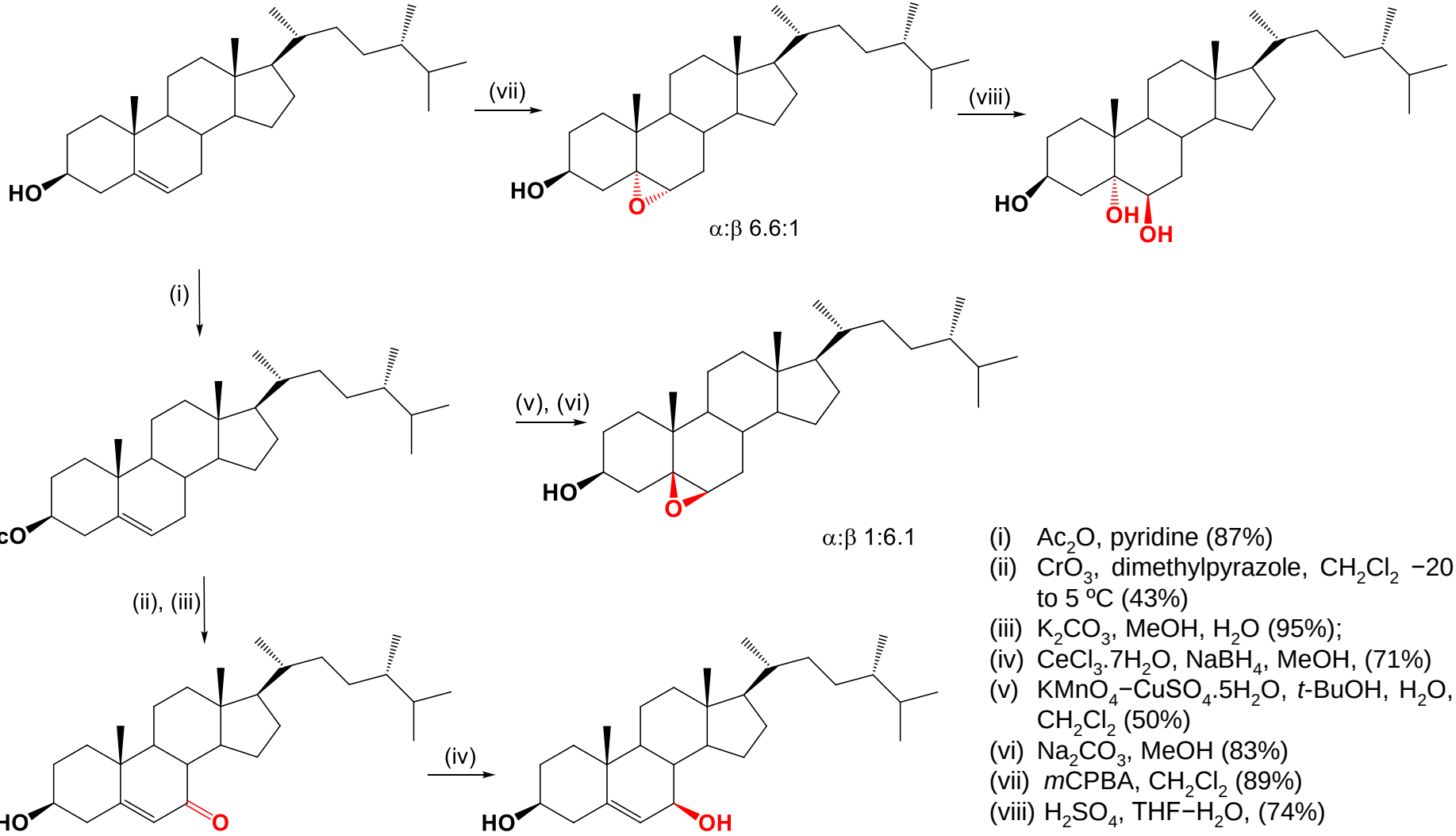
Synthesis of Dihydrobrassicasterol



Synthetic Challenges Overcome

- Aldehyde and Wittig salts need to be scrupulously dry as traces of water dramatically hinder the Wittig reaction.
- The success of the catalytic hydrogenation step depends on several variables:
  - Solvent (bulk and distilled ethanol/ethyl acetate)
  - Catalyst (Pd/C or PtO_2)
  - Pressure (50 – 100 psi)
  - Agitation method (shaken/stirred)
- Synthesis of Campesterol proves more challenging
  - Questions over purity of starting material and stereochemical integrity of some of the steps
  - Use of ^{1}H and ^{13}C NMR to identify problems

Oxidation of pure Dihydrobrassicasterol



(i) Ac_2O, pyridine (87%)
(ii) CrO_3, dimethylpyrazole, CH_2Cl_2 −20 to 5 ºC (43%)
(iii) K_2CO_3, MeOH, H_2O (95%);
(iv) $CeCl_3.7H_2O$, $NaBH_4$, MeOH, (71%)
(v) $KMnO_4$−$CuSO_4.5H_2O$, *t*-BuOH, H_2O, CH_2Cl_2 (50%)
(vi) Na_2CO_3, MeOH (83%)
(vii) *m*CPBA, CH_2Cl_2 (89%)
(viii) H_2SO_4, THF−H_2O, (74%)

Dihydrobrassicasterol oxide toxicity

Table 1. Cell Viability in U937 Cells Following Exposure for 24 h to 30, 60, or 120 μM DHB Oxides (95% Purity)[a]

compound	cell viability[b] mean	SE
Cholesterol Oxides		
30 μM α-epoxide	61.6**	2.5
30 μM β-epoxide	58.4**	9.9
30 μM 7-keto	64.1**	9.3
30 μM 7-β-OH	49.4**	10.4
Phytosterol Oxides		
30 μM α-epoxide	103.7	3.3
60 μM α-epoxide	92.2	8.5
120 μM α-epoxide	94.9	3.3
30 μM β-epoxide	81.2	4.4
60 μM β-epoxide	72.9*	3.7
120 μM β-epoxide	48. 9**	2.0
30 μM 7-keto	95.6	3.5
60 μM 7-keto	76.2	8.1
120 μM 7-keto	40.4**	7.3
30 μM 7-β-OH	117.1	14.6
60 μM 7-β-OH	39.3**	8.6
120 μM 7-β-OH	19.8**	6.0
30 μM triol	44.1**	13.2
60 μM triol	19.8**	5.2
120 μM triol	8.2**	1.7

Table 2. Percentage of Viable Cells Following Exposure for 24 h to 30, 60, or 120 μM of DHB Oxides (95% Purity)[a]

compound	cell viability[b] U937 cells mean	U937 cells SE	HepG2 cells mean	HepG2 cells SE
control (EtOH)	95.3	0.3	100.0	0.0
Cholesterol Oxides				
30 μM α-epoxide	89.1	2.4	95.8	3.6
30 μM β-epoxide	77.6**	2.8	73.3**	4.8
30 μM 7-keto	77.7**	1.7	95.5	4.4
30 μM 7-β-OH	78.9**	2.9	85.4	10.9
Phytosterol Oxides				
30 μM α-epoxide	90.1	1.1	100.1	2.0
60 μM α-epoxide	86.5	3.7	98.2	2.6
120 μM α-epoxide	89.4	2.6	103.6	3.1
30 μM β-epoxide	93.0	2.9	100.3	3.3
60 μM β-epoxide	89.5	4.0	99.9	3.9
120 μM β-epoxide	85.7	2.3	97.1	2.4
30 μM 7-keto	89.5	3.7	93.7	2.8
60 μM 7-keto	86.9	3.8	93.4	2.9
120 μM 7-keto	44.3**	5.7	89.5	2.8
30 μM 7-β-OH	85.5	4.0	93.8	3.6
60 μM 7-β-OH	64.6**	7.7	88.8	3.7
120 μM 7-β-OH	34.0**	1.7	81.7**	3.4
30 μM triol	72.7**	2.8	75.9**	3.6
60 μM triol	44.2**	7.2	63.4**	2.8
120 μM triol	7.0**	1.9	45.6**	2.1

Dihydrobrassicasterol oxide toxicity

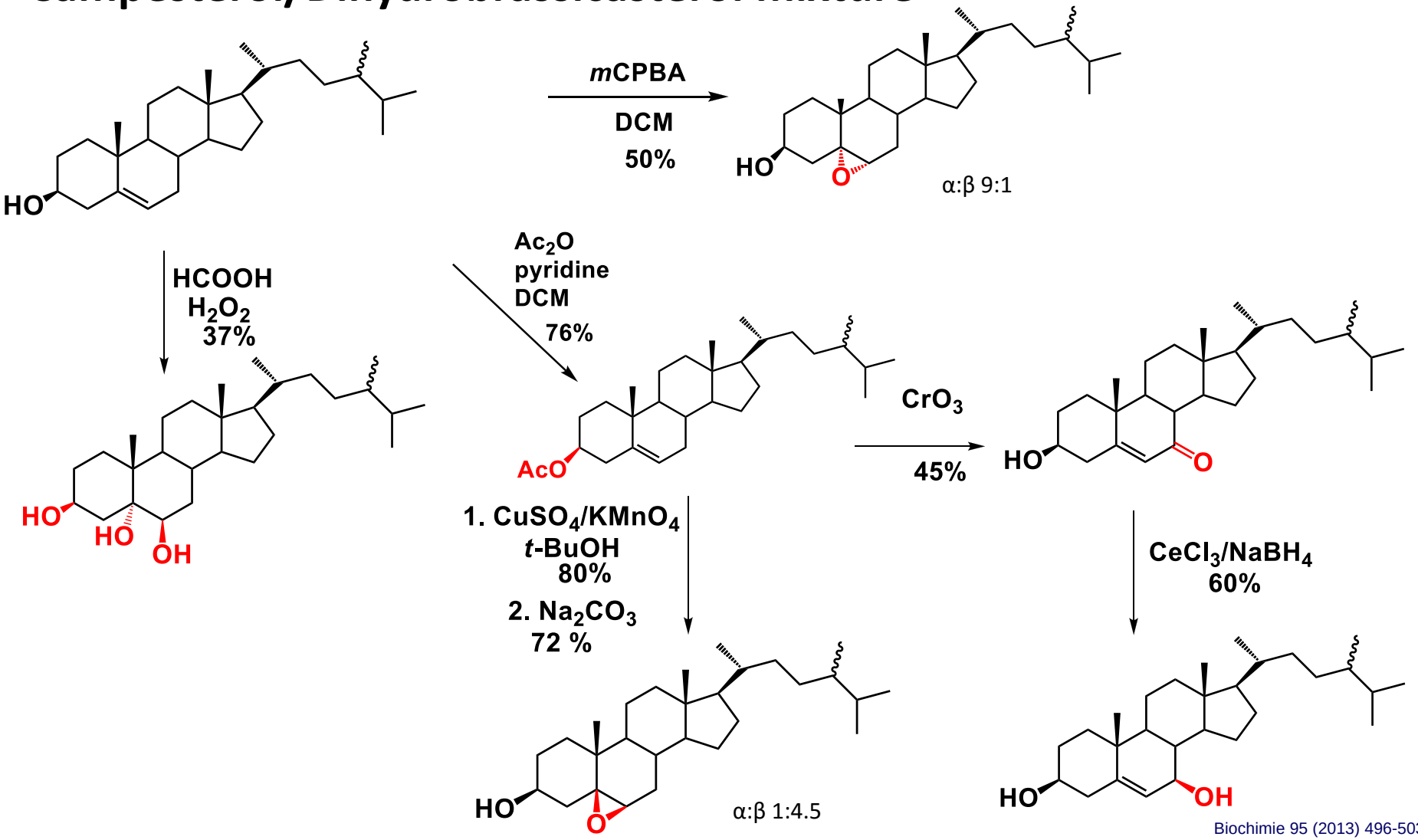
Table 3. Percentage of Condensed and Fragmented Nuclei in U937 Cells Following Exposure for 24 h to 30, 60, or 120 μM DHB Oxides (95% Purity)[a]

compound	% apoptosis[b] mean	SE
control (EtOH)	11.3	1.0
Cholesterol Oxides		
30 μM α-epoxide	21.3*	1.5
30 μM β-epoxide	28.1**	3.2
30 μM 7-keto	32.5**	2.7
30 μM 7-β-OH	27.5**	2.6
Phytosterol Oxides		
30 μM α-epoxide	18.6	1.7
60 μM α-epoxide	18.8	1.6
120 μM α-epoxide	21.7	2.6
30 μM β-epoxide	18.6	4.2
60 μM β-epoxide	20.4	1.3
120 μM β-epoxide	20.3	2.9
30 μM 7-keto	20.4	4.2
60 μM 7-keto	31.5**	3.1
120 μM 7-keto	37.1**	5.4
30 μM 7-β-OH	21.4	2.0
60 μM 7-β-OH	23.6*	1.9
120 μM 7-β-OH	27.7**	4.4
30 μM triol	18.3	3.7
60 μM triol	19.3	1.2
120 μM triol	18.9	5.1

Table 4. Caspase Activity Following Exposure for 24 h to 30, 60, or 120 μM DHB Oxides (95% Purity)[a]

compound	caspase act. (fold increase rel to control ethanol)[b] U937 cells mean	SE	HepG2 cells mean	SE
Cholesterol Oxides				
30 μM α-epoxide	1.2	0.1	1.1	0.1
30 μM β-epoxide	2.0*	0.4	1.3	0.2
30 μM 7-keto	1.9*	0.2	1.0	0.1
30 μM 7-β-OH	1.4	0.2	1.2	0.2
Phytosterol Oxides				
30 μM α-epoxide	1.0	0.1	1.1	0.1
60 μM α-epoxide	1.1	0.1	1.1	0.1
120 μM α-epoxide	1.5	0.3	1.0	0.1
30 μM β-epoxide	0.9	0.1	0.9	0.0
60 μM β-epoxide	1.3	0.2	1.0	0.1
120 μM β-epoxide	1.8	0.5	1.1	0.1
30 μM 7-keto	1.0	0.0	0.9	0.1
60 μM 7-keto	1.6	0.3	0.9	0.0
120 μM 7-keto	1.5	0.2	1.0	0.1
30 μM 7-β-OH	1.3	0.1	0.9	0.0
60 μM 7-β-OH	2.3*	0.3	1.2	0.1
120 μM 7-β-OH	3.0**	0.5	1.2	0.2
30 μM triol	1.1	0.2	1.5	0.1
60 μM triol	1.3	0.2	2.5**	0.2
120 μM triol	1.6	0.5	4.0**	0.4

Oxidation of phytosterols – Campesterol/Dihydrobrassicasterol mixture



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Campesterol/Dihydrobrassicasterol oxide toxicity

Table 1
Percent viable U937 cells following exposure to 3DHB:1CMP or 2CMP:1DHB oxides at concentrations of 30 µM, 60 µM or 120 µM for 24 h, as determined by the fluorescein diacetate (FDA/EtBr) assay.

	3DHB:1CMP (**d**) mean ± se	2CMP:1DHB (**c**) mean ± se
Control	95.3 ± 0.3	97.5 ± 0.4
30 µM α-epoxide (**8**)	87.5 ± 4.3	95.6 ± 0.9
60 µM α-epoxide (**8**)	89.4 ± 3.0	96.0 ± 1.5
120 µM α-epoxide (**8**)	79.2 ± 2.9	88.7 ± 2.0
30 µM β-epoxide (**7**)	84.7 ± 5.9	96.0 ± 0.6
60 µM β-epoxide (**7**)	92.3 ± 1.6	94.6 ± 0.4
120 µM β-epoxide (**7**)	85.8 ± 3.4	62.3 ± 5.4*
30 µM 7-keto (**4**)	90.3 ± 3.1	95.0 ± 1.2
60 µM 7-keto (**4**)	77.3 ± 5.5	82.8 ± 3.6
120 µM 7-keto (**4**)	30.2 ± 2.8*	68.8 ± 1.4*
30 µM 7β-OH (**5**)	88.5 ± 4.6	63.0 ± 3.1*
60 µM 7β-OH (**5**)	69.0 ± 8.8*	36.2 ± 1.6*
120 µM 7β-OH (**5**)	38.5 ± 8.5*	27.3 ± 2.2*
30 µM 3,5,6-triol (**9**)	62.9 ± 2.2*	63.2 ± 4.4*
60 µM 3,5,6-triol (**9**)	34.0 ± 12.0*	45.5 ± 2.8*
120 µM 3,5,6-triol (**9**)	11.5 ± 3.5*	32.6 ± 3.1*

Table 2
Cell viability in U937 cells following exposure to 3DHB:1CMP or 2CMP:1DHB oxides at concentrations of 30 µM, 60 µM or 120 µM for 24 h, as determined by the MTT assay.

	3DHB:1CMP (**d**) mean ± se	2CMP:1DHB (**c**) mean ± se
30 µM α-epoxide (**8**)	0.96 ± 0.09	0.74 ± 0.03*
60 µM α-epoxide (**8**)	0.93 ± 0.07	0.67 ± 0.05*
120 µM α-epoxide (**8**)	0.52 ± 0.05*	0.35 ± 0.06*
30 µM β-epoxide (**7**)	0.71 ± 0.07	0.86 ± 0.04*
60 µM β-epoxide (**7**)	0.68 ± 0.05*	0.81 ± 0.04*
120 µM β-epoxide (**7**)	0.66 ± 0.04*	0.35 ± 0.01*
30 µM 7-keto (**4**)	0.80 ± 0.06	0.55 ± 0.02*
60 µM 7-keto (**4**)	0.60 ± 0.07*	0.11 ± 0.00*
120 µM 7-keto (**4**)	0.12 ± 0.03*	0.04 ± 0.01*
30 µM 7β-OH (**5**)	0.92 ± 0.14	0.14 ± 0.07*
60 µM 7β-OH (**5**)	0.55 ± 0.08*	0.02 ± 0.02*
120 µM 7β-OH (**5**)	0.20 ± 0.04*	0.00 ± 0.01*
30 µM 3,5,6-triol (**9**)	0.49 ± 0.11*	0.25 ± 0.04*
60 µM 3,5,6-triol (**9**)	0.15 ± 0.03*	0.04 ± 0.02*
120 µM 3,5,6-triol (**9**)	0.05 ± 0.01*	0.03 ± 0.02*

Table 3
Viability in HepG2 cells following exposure to 3DHB:1CMP or 2CMP:1DHB oxides at concentrations of 30 µM, 60 µM or 120 µM for 24 h expressed as percent control, as determined by the neutral red uptake assay.

	3DHB:1CMP (**d**) mean ± se	2CMP:1DHB(**c**) mean ± se
30 µM α-epoxide (**8**)	97.1 ± 5.3	97.9 ± 4.1
60 µM α-epoxide (**8**)	99.3 ± 5.8	90.9 ± 4.0
120 µM α-epoxide (**8**)	99.4 ± 4.2	77.9 ± 1.9*
30 µM β-epoxide (**7**)	105.8 ± 4.4	87.5 ± 2.0
60 µM β-epoxide (**7**)	100.3 ± 4.2	92.0 ± 1.3
120 µM β-epoxide (**7**)	87.5 ± 4.9	89.5 ± 4.8
30 µM 7-keto (**4**)	92.7 ± 3.6	79.8 ± 4.2*
60 µM 7-keto (**4**)	82.6 ± 4.3*	80.0 ± 10.1*
120 µM 7-keto (**4**)	65.7 ± 3.4*	78.3 ± 4.8*
30 µM 7β-OH (**5**)	94.6 ± 3.0	77.0 ± 3.0*
60 µM 7β-OH (**5**)	88.4 ± 1.6	95.6 ± 4.8
120 µM 7β-OH (**5**)	80.7 ± 1.7*	45.8 ± 4.3*
30 µM 3,5,6-triol (**9**)	79.2 ± 5.4*	52.3 ± 7.4*
60 µM 3,5,6-triol (**9**)	69.3 ± 4.1*	48.3 ± 5.5*
120 µM 3,5,6-triol (**9**)	45.0 ± 4.2*	41.1 ± 5.0*

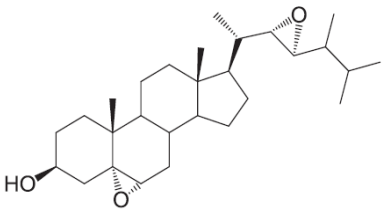
Table 4
Apoptotic nuclei in U937 cells following exposure to 3DHB:1CMP or 2CMP:1DHB oxides at concentrations of 30 µM, 60 µM or 120 µM for 24 h, as determined by staining with Hoechst 33342.

	3DHB:1CMP (**d**) mean ± se	2CMP:1DHB (**c**) mean ± se
Control	1.00 ± 0.08	1.00 ± 0.08
30 µM α-epoxide (**8**)	1.48 ± 0.20	0.86 ± 0.32
60 µM α-epoxide (**8**)	1.85 ± 0.30	1.59 ± 0.23
120 µM α-epoxide (**8**)	2.33 ± 0.16	2.77 ± 0.45
30 µM β-epoxide (**7**)	1.54 ± 0.40	1.50 ± 0.27
60 µM β-epoxide (**7**)	1.65 ± 0.22	2.23 ± 0.27
120 µM β-epoxide (**7**)	1.93 ± 0.30	7.23 ± 0.91*
30 µM 7-keto (**4**)	2.12 ± 0.37	3.04 ± 0.36
60 µM 7-keto (**4**)	3.47 ± 0.58*	3.95 ± 0.64
120 µM 7-keto (**4**)	3.53 ± 0.42*	4.86 ± 1.73*
30 µM 7β-OH (**5**)	1.97 ± 0.34	5.86 ± 2.00*
60 µM 7β-OH (**5**)	2.62 ± 0.24*	6.54 ± 1.86*
120 µM 7β-OH (**5**)	3.15 ± 0.36*	6.82 ± 0.18*
30 µM 3,5,6-triol (**9**)	2.05 ± 0.12	2.91 ± 0.36
60 µM 3,5,6-triol (**9**)	2.65 ± 0.50*	4.45 ± 0.27
120 µM 3,5,6-triol (**9**)	3.55 ± 0.12*	3.23 ± 0.73

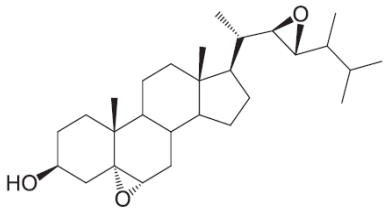
Bisepoxide toxicity

Table 5
Cytotoxicity in U937 and HepG2 cells and apoptotic nuclei in U937 cells following exposure to 5,6,22,23-Diepoxycampestane (Bisepoxide **12c**) at concentrations of 30 μM, 60 μM or 120 μM for 24 h.

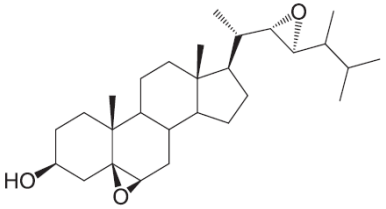
	Viability (U937 cells) mean ± se	Viability (HepG2 cells) mean ± se	Apoptosis (U937 cells) mean ± se
30 μM Bisepoxide (**12c**)	11.7 ± 5.2*	48.4 ± 8.2*	4.45 ± 0.91*
60 μM Bisepoxide (**12c**)	1.3 ± 1.8*	0.0 ± 5.6*	0.59 ± 0.27
120 μM Bisepoxide (**12c**)	0.0 ± 0.0*	0.0 ± 5.4*	0.41 ± 0.14



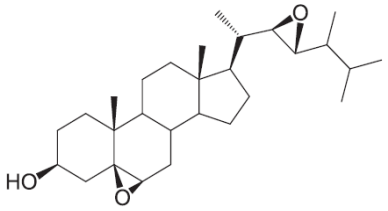
12c$_1$ 5α,6α,22β,23β



12c$_2$ 5α,6α,22α,23α



12c$_3$ 5β,6β,22β,23β



12c$_4$ 5β,6β,22α,23α

Conclusions

- Reported the synthesis and toxicity of stigmasterol oxides
  - Significance of the 22,23-oxides
- First reported synthesis of Dihydrobrassicasterol
  - Capable of multigram synthesis of this key phytosterol
- Synthesis of Dihydrobrassicasterol and Campesterol enriched mixtures
- Panel of oxides in each series fully characterised and synthesised
- Toxicity of oxides evaluated
  - Obvious concentration dependent toxicity evident for POPs
  - Most toxic variants oxidised on side chain
  - Oxysterol toxicity hypothesis proven – long term studies

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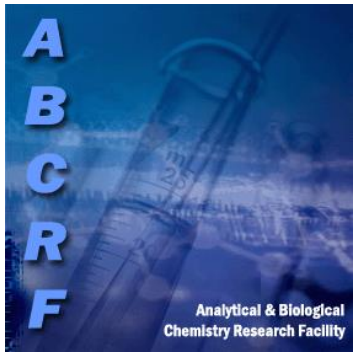
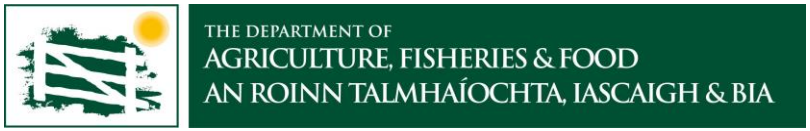
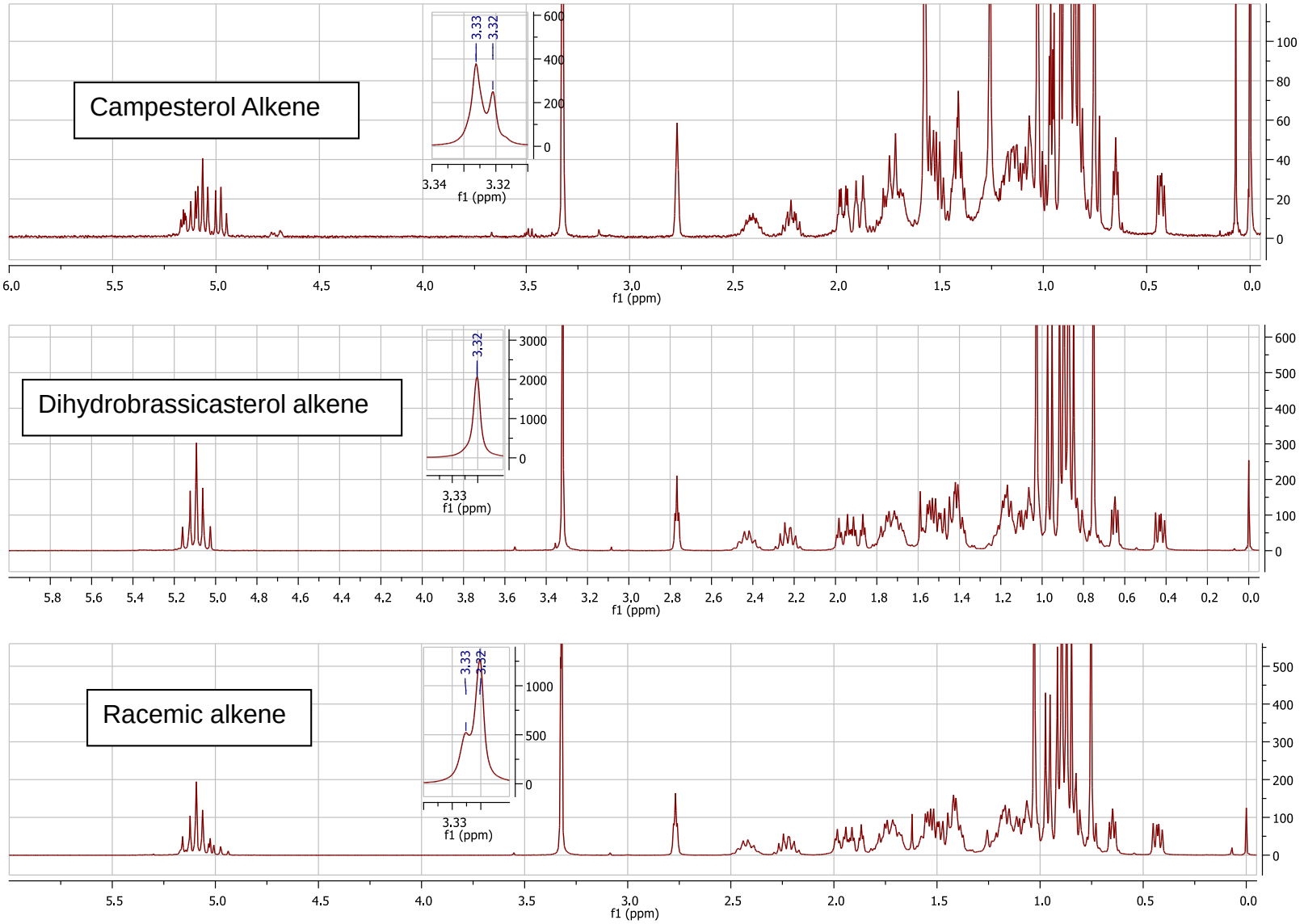
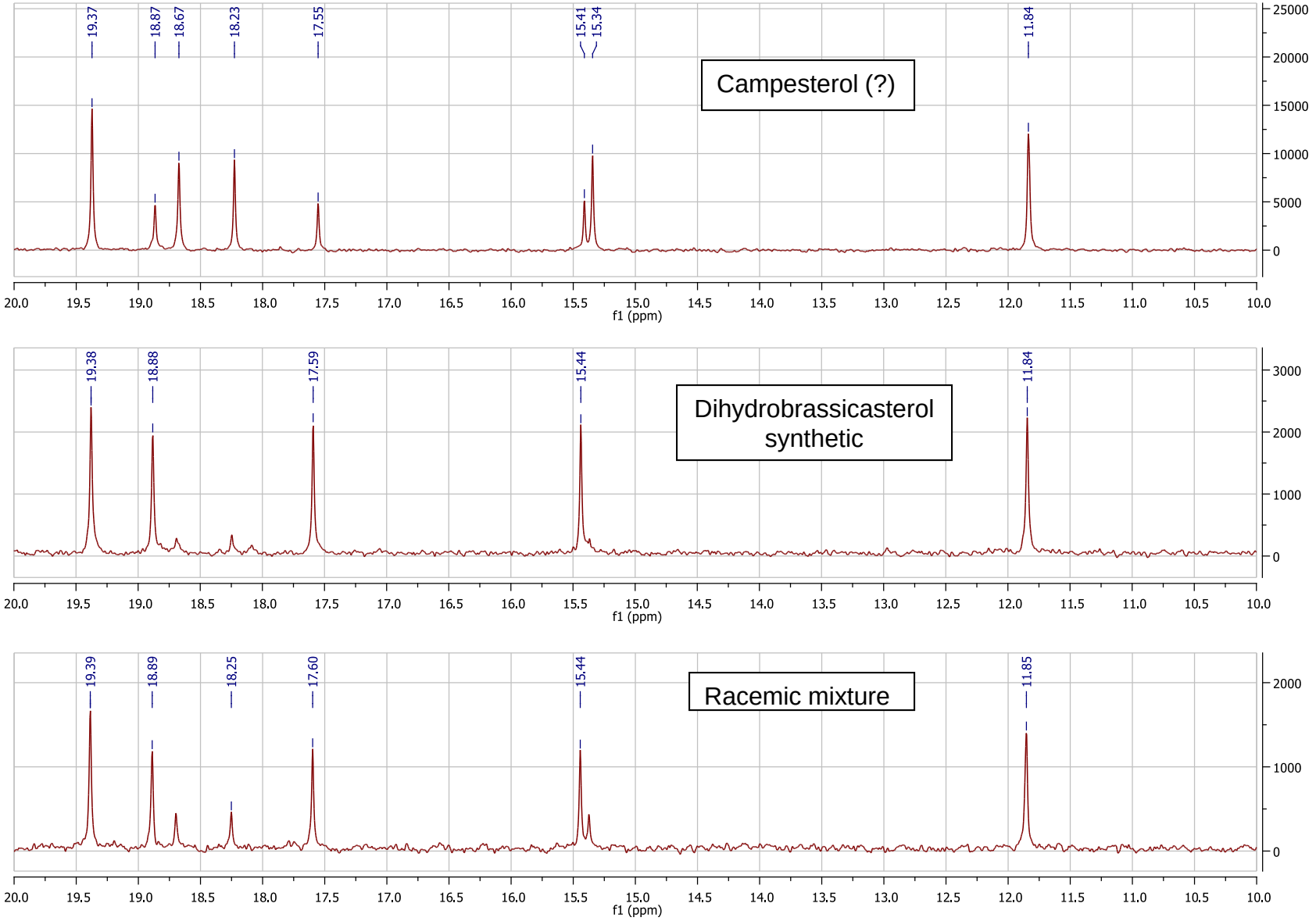




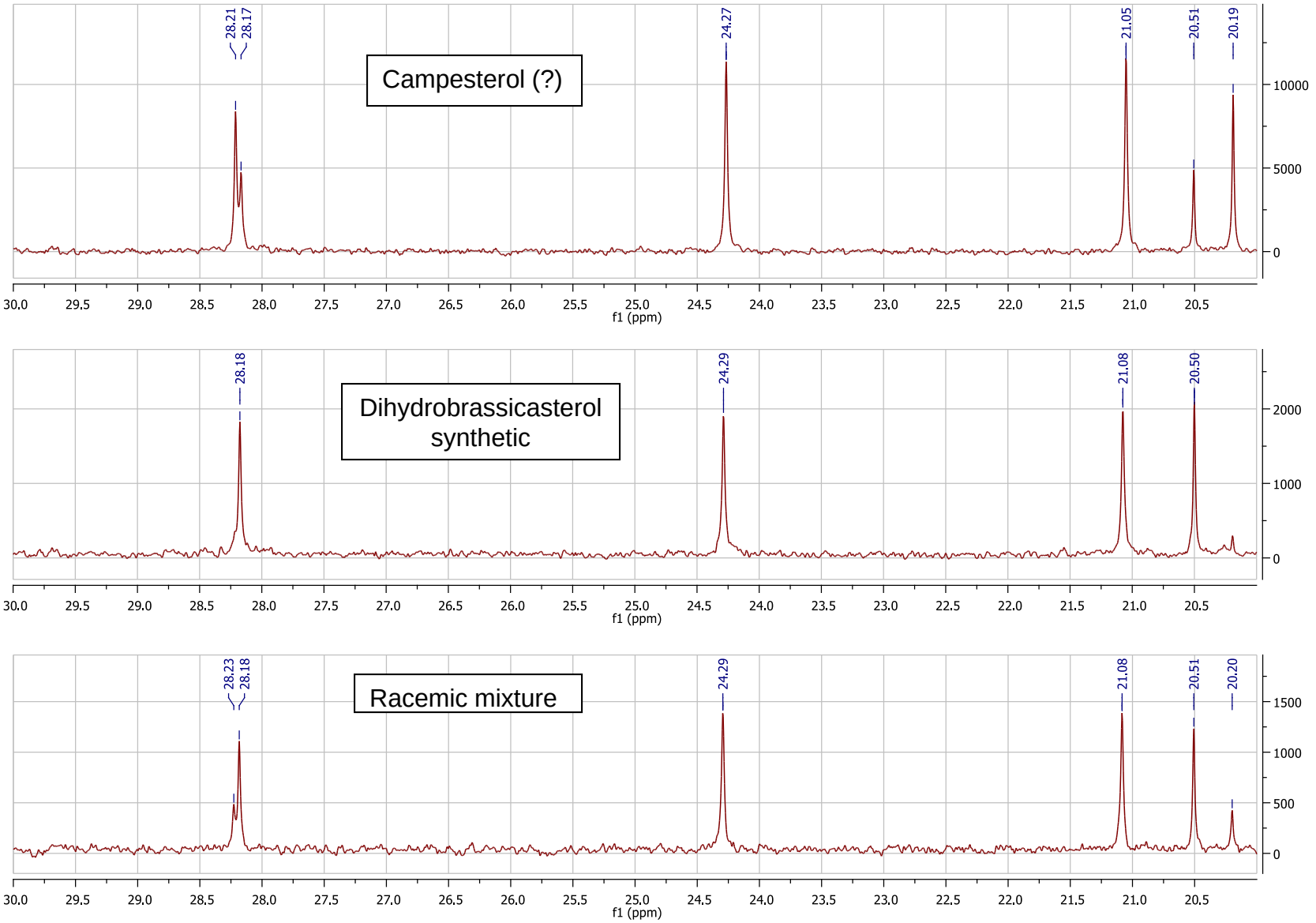
600MHz ^{1}H NMR comparison of the Wittig reaction products (alkenes **14, 29 and 38**) on route to Dihydrobrassicasterol and Campesterol



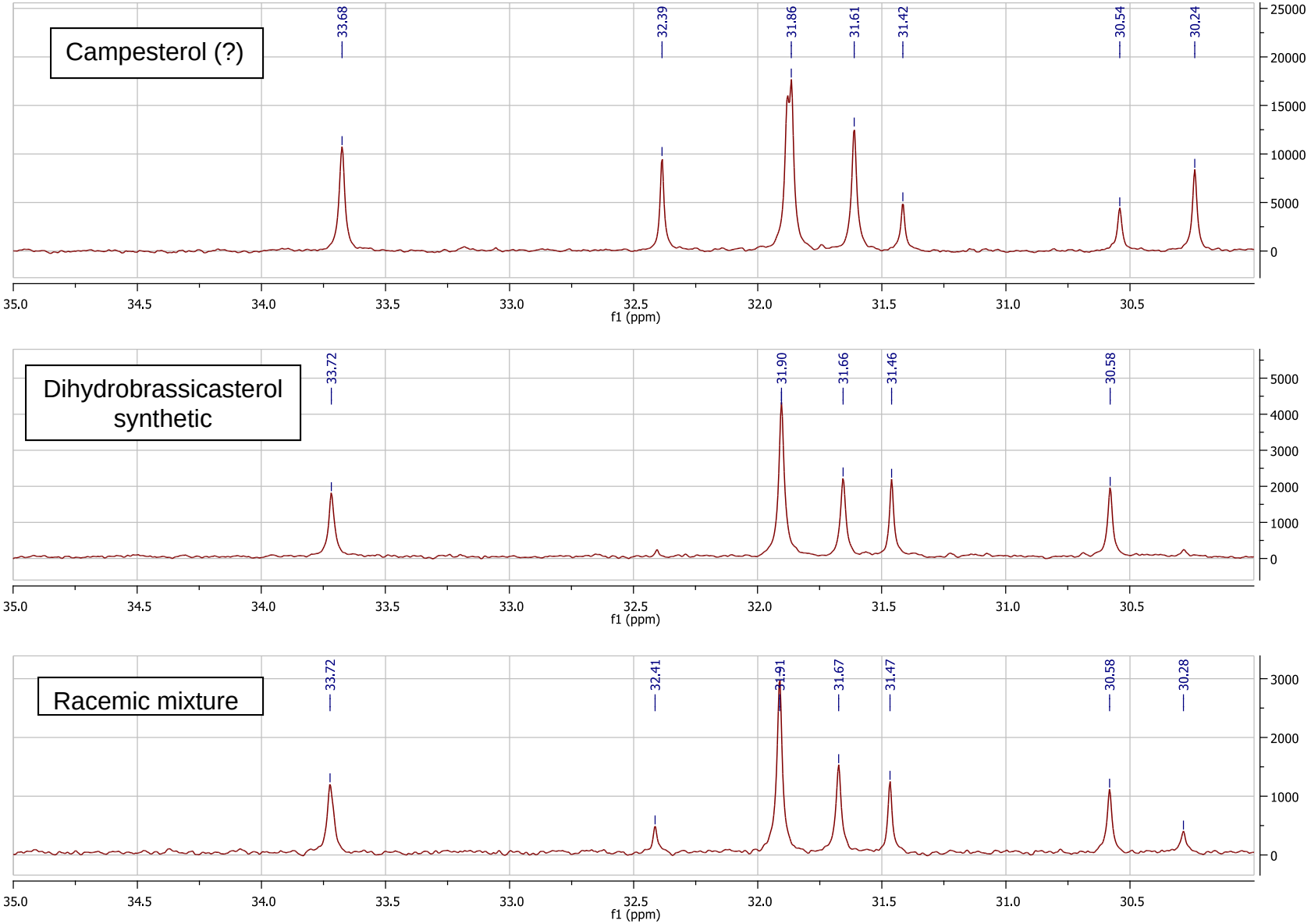
150MHz ^{13}C NMR comparison of Dihydrobrassicasterol and Campesterol between 10-20ppm



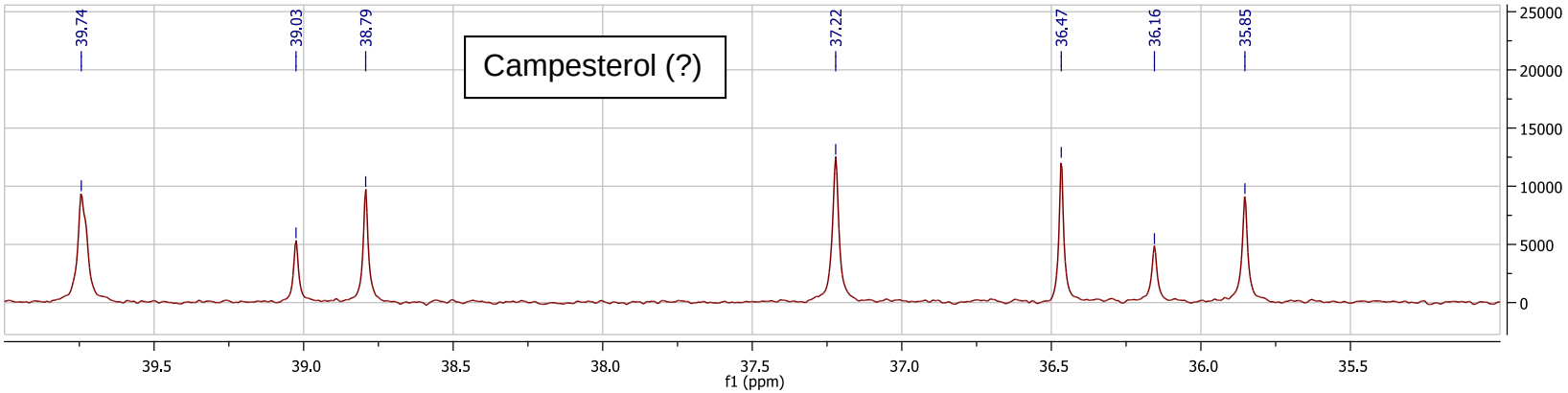
150MHz ^{13}C NMR comparison of Dihydrobrassicasterol and Campesterol between 20-30ppm

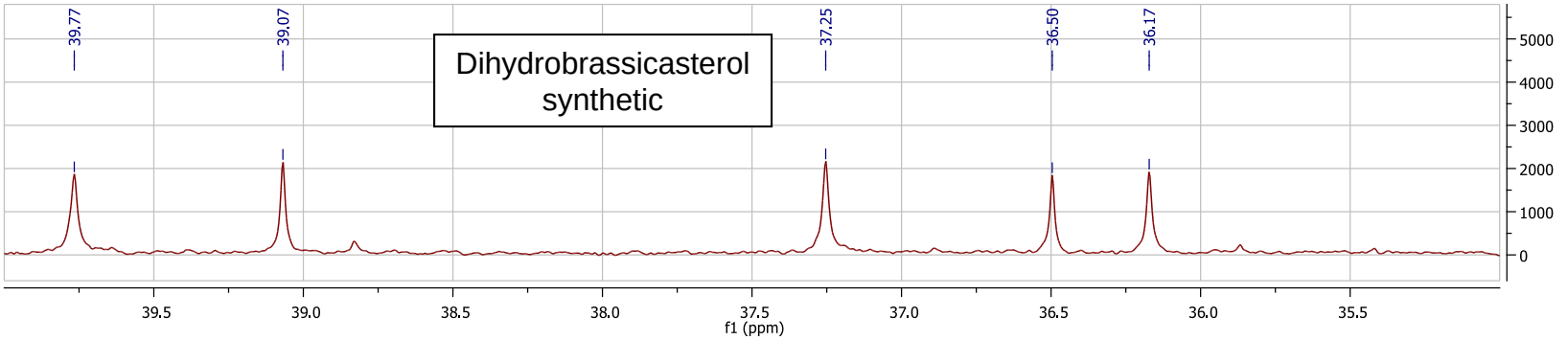


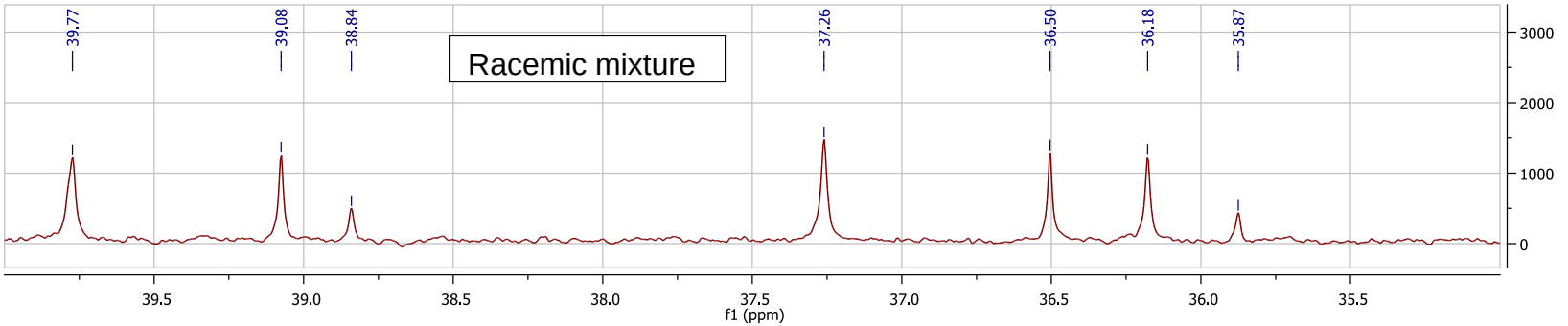
150MHz ^{13}C NMR comparison of Dihydrobrassicasterol and Campesterol between 30-35ppm



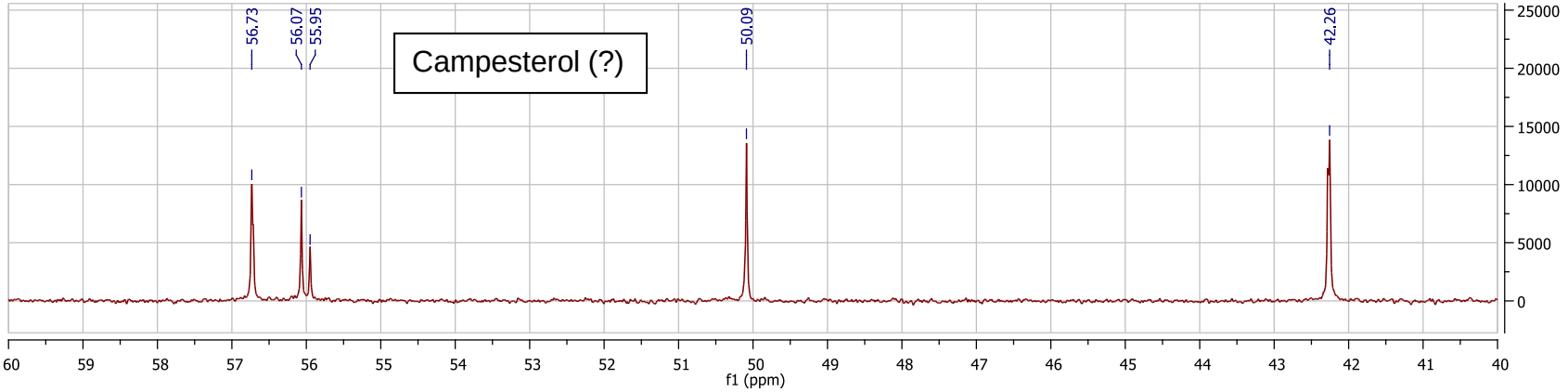
150MHz ^{13}C NMR comparison of Dihydrobrassicasterol and Campesterol between 35-40ppm

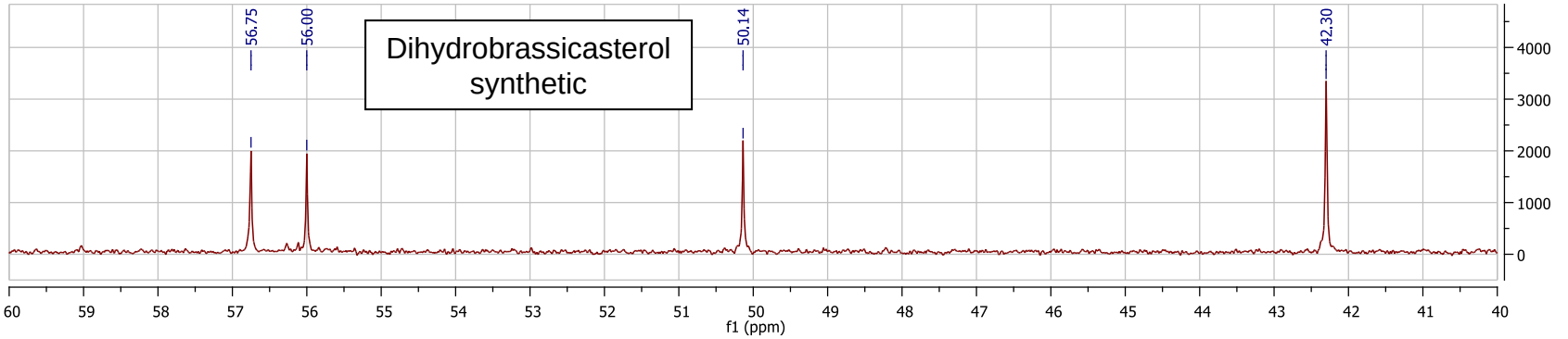


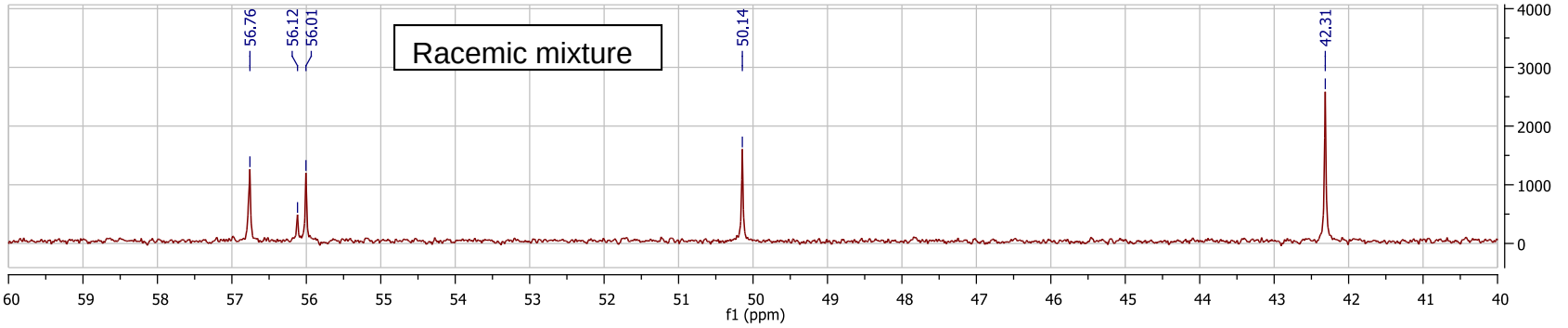




150MHz ^{13}C NMR comparison of Dihydrobrassicasterol and Campesterol between 40-60ppm







150MHz ^{13}C NMR comparison of Dihydrobrassicasterol and Campesterol between 60-145ppm