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The Seleno-acetal Route to 1α-Hydroxy-vitamin D Analogues: Synthesis of 24-Oxa-1α-hydroxy-vitamin D_3, A Useful Vitamin D Metabolism Probe

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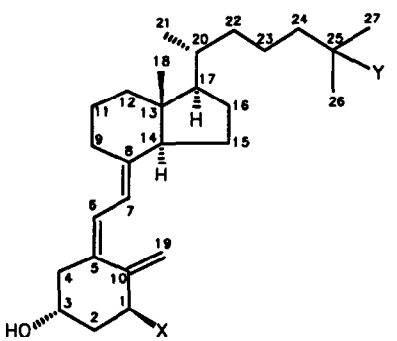
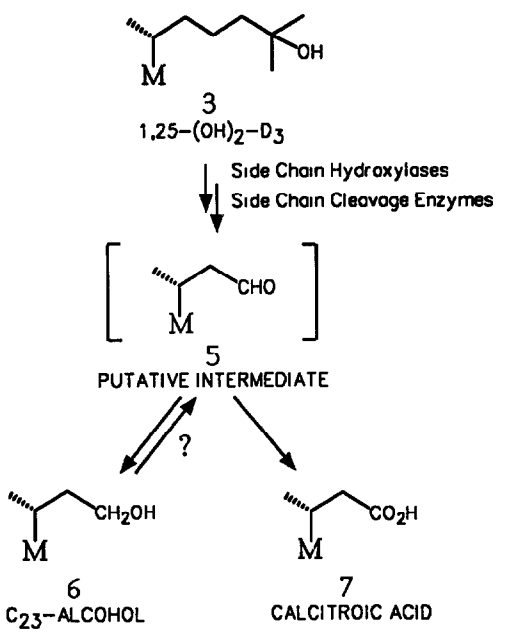
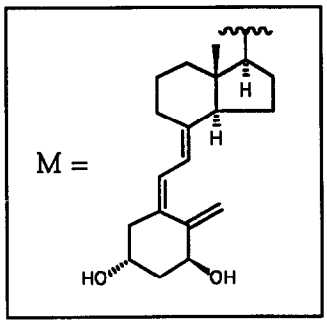
Abstract. Alkylation of the lithio-demethylseleno-derivative of seleno-acetal **11** with chloromethyl isopropyl ether [shown by NMR to give the (22*S*)-methylseleno-compound **13a** as the major diastereoisomer] is the key reaction in the synthesis of the 24-oxa analogue (MC 1090, **8**) of 1α-hydroxyvitamin D_3 (**4**) The metabolism of **8** to calcitroic acid (**7**) is demonstrated *in vitro* in a hepatocyte cell (Hep 3B) model This supports the hypothesis that **8** can undergo enzymatic 25-hydroxylation analogous to the activation of **4**, or a similar side chain hydroxylation, but then short cuts the target cell side chain cleavage pathway taking 1α,25-dihydroxyvitamin D_3 to **7**.

Vitamin D_3 (**1**), formed naturally in the skin by (photo- followed by thermal-) isomerisation of 7-dehydrocholesterol, is transported bound to a protein in the plasma to the liver where 25-hydroxylation occurs, the first step of its metabolic activation required for function Further metabolism of 25-hydroxyvitamin D_3 (25-OH-D_3, **2**) to the hormonally active 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2$-D_3, **3**) takes place in the kidney and is under strict feedback regulation The interaction of 1,25-$(OH)_2$-D_3 with a specific receptor present in the classical target cells (intestine, bone, kidney) initiates a cascade of events resulting in the transport of calcium ions necessary for homeostasis [1] Target cell catabolism of 1,25-$(OH)_2$-D_3 (**3**) by 1,25-$(OH)_2$-D_3 -inducible enzymes is believed to proceed sequentially *via* the 24(*R*)-hydroxy-, 24-oxo-, and 23(*S*)-hydroxy-24-oxo-derivatives to the inactive, side chain cleaved C_{23} compound calcitroic



1	X = Y = H	Vitamin D_3
2	X = H, Y = OH	25-OH-D_3
3	X = Y = OH	1,25-$(OH)_2$-D_3
4	X = OH, Y = H	1α-OH-D_3



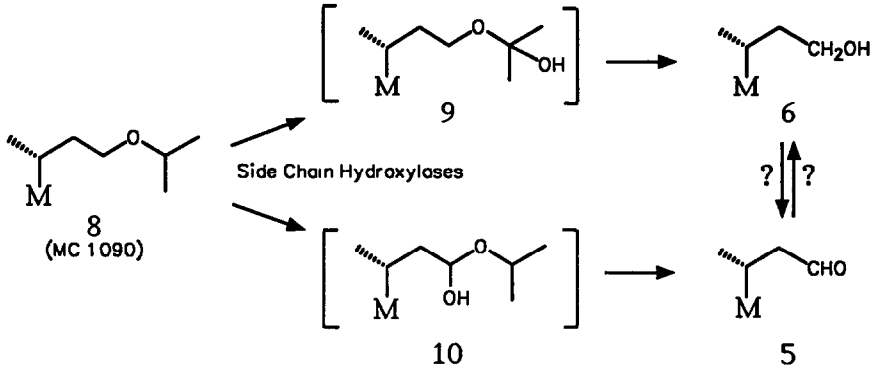


Scheme 1 Target cell catabolism of 1,25-$(OH)_2$-D_3 (**3**)

acid (**7**) (Scheme 1), which is transported to the liver and excreted in the bile [2] The C_{23} alcohol (**6**) has also been identified on this pathway and suggested to be the precursor of calcitroic acid [3] Our experiments with a synthetic sample of the putative pivotal intermediate C_{23} aldehyde (**5**) indicate that the alcohol is probably a side product of the pathway, though both can be converted by a 1,25-$(OH)_2$-D_3-inducible enzyme to calcitroic acid [4]

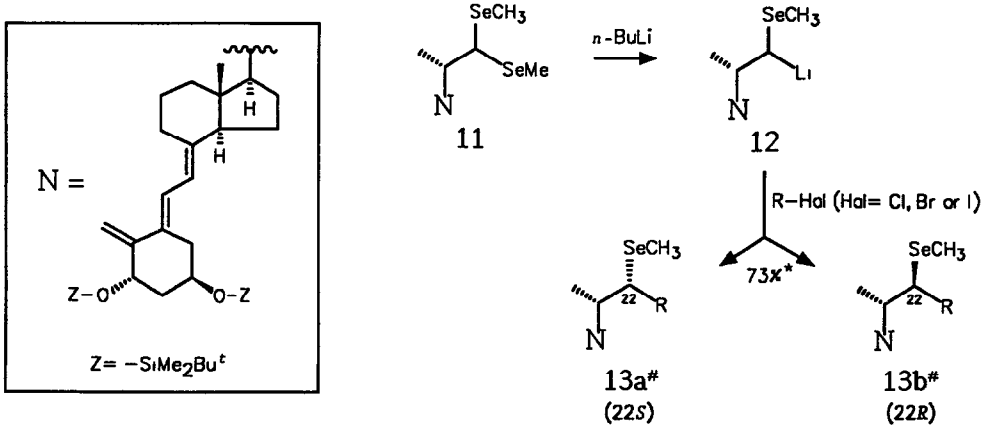
The liver vitamin-D_3-25-hydroxylase has been shown to possess some variability in its substrate selectivity Thus, the synthetic analogue 1α-hydroxyvitamin D_3 (1α-OH-D_3, **4**) is hepatically activated to 1,25-$(OH)_2$-D_3 (**3**) (without feedback control) and is a therapeutically important prodrug for the hormone [5] A modified side chain can also be hydroxylated, as is the case with vitamin D_2 [6] A human hepatocyte (Hep 3B) cell model has been shown to provide an accurate *in vitro* reflection of the *in vivo* 25-hydroxylation of vitamin D_3[7] and analogues (including **4**) and has also provided an insight into the possibility of varying the hydroxylation site by structural modification For example, the 26,27-cyclo-derivative of 1α-OH-D_3 (MC 969) is exclusively 24-hydroxylated by this system [8]

To probe further the details of vitamin D catabolism we have designed an analogue, the 24-oxa-derivative of 1α-OH-D_3 (**8**, MC 1090 = 24-oxa-**4**), which, if a substrate for the vitamin-D-hydroxylase, can short cut the calcitroic acid pathway Thus, 25-hydroxylation of **8** would give the C_{23} alcohol (**6**) directly from decomposition of the unstable hemi-acetal intermediate (**9**) (Scheme 2) An alternative possibility, 23-hydroxylation to **10**, would give the C_{23} aldehyde (**5**) Either of these side chain cleaved compounds could then be converted to **7** We report here a synthesis of MC 1090 together with the results of the *in vitro* metabolism studies [9] The metabolism studies have been published in abstract form.[10]



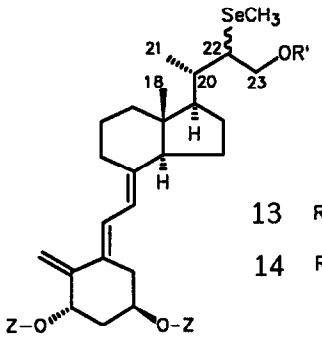
Scheme 2 Proposed metabolic side chain cleavage pathways for MC 1090 (8)

Alkylation of the lithio-demethylseleno-derivative (12) of seleno-acetal 11 to give two series of 22-methylseleno-compounds (*cf.* Scheme 3) constitutes the key reaction in the synthesis of a variety of side chain modified analogues of 1,25-$(OH)_2$-D_3 (3),[11-15] including 1α-OH-D_3 (4), for which the alkylating agent (R-Hal) is isoamyl bromide [11] A corresponding alkylation with chloromethyl isopropyl ether (as illustrated) gave the anticipated intermediates 13 for the MC 1090 synthesis, though in this case the diastereoisomers at C-22 were chromatographically inseparable, and recrystallisation only marginally enriched the mixture, which therefore had to be used in the subsequent steps as such The kinetic ratio of the 22-epimers in 13 was readily determined as 3 2 from the NMR-spectra (see Experimental Section) A modest selectivity in this reductive alkylation of 11 was expected on the basis of our experience with alkylations using unactivated primary alkyl halides[11] and also a reductive formylation[13] and hydroxy-alkylation[14] of 11, for



Scheme 3.

* R-Hal = Cl-$CH_2OCH(CH_3)_2$, # R = -$CH_2OCH(CH_3)_2$



13 R' = $CH(CH_3)_2$

14 R' = H

Z = $-SiMe_2Bu^t$

which reactions we were able to demonstrate the preferential formal substitution of the pro-*R* selenomethyl group. For the conversion of **11** to **13** we can convincingly assign the same stereoselectivity by NMR comparison (see Table) of the product mixture with the previously described[13] alcohols **14**, whose configurations have been deduced. The juxtaposition of data shown clearly allows correlation of 22*S*-**14** with the major isomer **13** (thus assigned the structure **13a**) and of 22*R*-**14** with the minor isomer (*viz.* **13b**).[16]

Table. Chemical Shifts[a] for Selected Signals in the NMR Spectra of Compounds 13 and 14.[b]

	C-18	*C*-21	*C*-20	*C*-23	$SeCH_3$
13	11 5 / 12.2	15 6 / 14.6	40.3 / 36 4	69.1 / 70.8	4.5 / 4.1
$\Delta\delta$[c]	-0.7	1.0	3.9	-1.7	0.4
(22*S*/*R*)-14	11.5 / 12 2	16 1 / 15.3	41 1 / 37 6	61 2 / 64.8	4.8 / 4.4
$\Delta\delta$[d]	-0.7	0.8	3 5	-3 6	0.4

	18-H_3	21-H_3	22-*H*	$SeCH_3$
13	0.55 / 0 58	1.03 / 0 9[e]	3 15 / 2 97	2 06 / 2.02
Appearance[f]	s s	d (6 8) ?	m bt (7.5)	s s
(22*S*/*R*)-14	0 55 / 0.59	1.03 / 0 9[e]	3 08 / 2 98	2 06 / 2.02
Appearance[f]	s s	d (6 9) ?	m bt (7.5)	s s

a δ ppm, 300 (^1H) or 75 5 (^{13}C) MHz, solvent $CDCl_3$, ref $CHCl_3$ = 7 26 or $CDCl_3$ = 76 8

b Data for **13** (major / minor epimers) are abstracted from the Experimental Section Data for **14** were obtained from the spectra of the available[13] pure 22*S*-**14** and *ca* 1 1 mixture of 22*S*-**14** and 22*R*-**14**

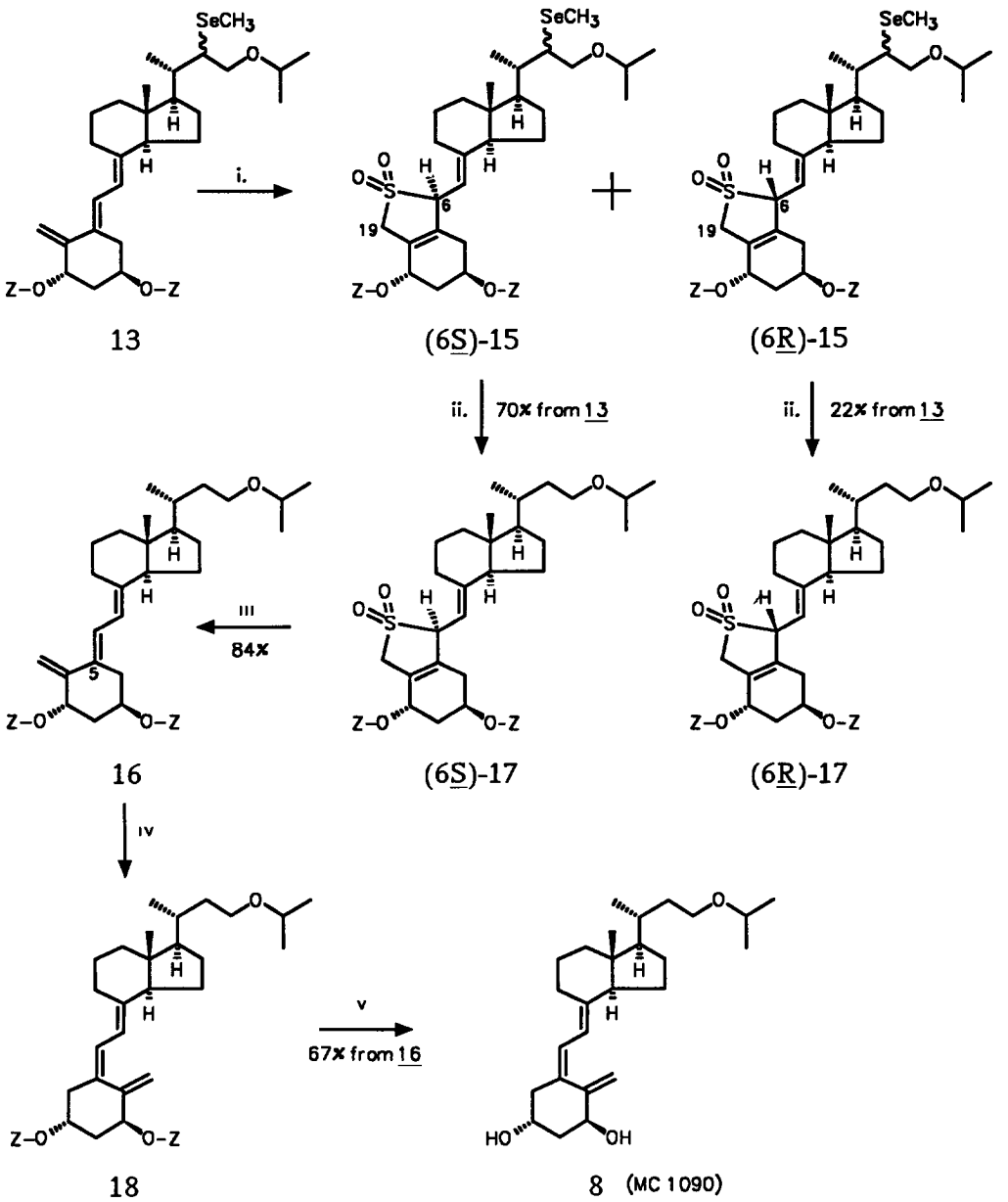
c Chemical shift difference between major and minor signals

d Chemical shift difference between the signals in 22*S*-**14** and 22*R*-**14**

e Approximate position signal partially obscured

f Abbreviations s= singlet, d= doublet, bt= broad triplet, m= multiplet, (Coupling constant *J* in Hz)

Following the established methodology,[11] for the radical deselenation reaction, the conjugated triene moiety of **13** was first masked as the cycloaddition products (**15**) formed with sulphur dioxide (which adds across C-6 and C-19 to give a *ca* 3·1 mixture of 6*S* and 6*R* adducts) (Scheme 4) Photo-induced reduction with tributyltin hydride then cleanly gave the SO_2-adducts **17** these were separated chromatographically during purification Thermal cheletropic extrusion of SO_2 from either isomer **17** regenerated the 5*E*-vitamin D system in **16**, and the synthesis of MC 1090 (**8**) was completed by the standard sequence of triplet sensitised 5*E* to 5*Z* photo-isomerisation[17] (to **18**) followed by desilylation with fluoride



Scheme 4 (Z = -SiMe$_2$Bu^t)

i. SO_2; ii *n*-Bu_3SnH - hν; separate, iii $NaHCO_3$ (boiling EtOH), iv anthracene - hν; v. *n*-Bu_4N^+ F^-.

Incubation of MC 1090 (10 μg/mL) with Hep 3B cells for 48 h followed by methanol/dichloromethane extraction allowed discernment of both lipid- and aqueous-soluble metabolites.[18] Analysis of the lipid-soluble fraction on HPLC with diode-array detection (for details of the methods, see ref 8) clearly showed

the presence of 5 compounds displaying the characteristic vitamin D triene chromophore. One of these, formed in substantial amounts (*ca.* 60 ng/10^7 cells/day), was identified (after isolation) as the C_{23} alcohol **6** by direct comparison with authentic material[13] (comigration on two different HPLC systems and mass spectrometry both with and without per-trimethylsilylation) Analysis of the aqueous-soluble fraction from the extraction (for details of the methods, see ref. 2) led to the measurement of large amounts (*ca* 140 ng/10^7 cells/day) of calcitroic acid (**7**) (comigrating on reverse-phase HPLC with authentic material[13]), conclusively identified by isolation and comparison (HPLC and MS) of its methylated (diazomethane) derivative with authentic[13] methyl calcitroate. The aldehyde **5** was not a detectable metabolite

The identification of both the C_{23} alcohol (**6**) and calcitroic acid (**7**) as metabolites of MC 1090 is in accord with our original hypothesis (Scheme 2) on the breakdown of side chain hydroxylated MC 1090, though we have not resolved the question of whether the two metabolites lie on the same or on divergent pathways. If both compounds result from exclusive 25-hydroxylation, then the alcohol is an intermediate, while this is not necessarily the case if 23-hydroxylation is operative. Although further details need clarifying, the results so far have provided the first demonstration that the terminal steps of calcitroic acid production can take place in non-target cells for the vitamin D hormone.

EXPERIMENTAL SECTION

General. Reactions were performed routinely under a nitrogen atmosphere Petroleum ether refers to the pentane fraction Organic solutions were dried over anhyd. $MgSO_4$ Column chromatography was performed on Merck Kieselgel 60 using the "flash" technique Analytical TLC (to which R_f values refer) was performed on Merck plates pre-coated with silica gel 60 F_{254}. Melting points were detemined with a Buchi-Tottoli apparatus and are uncorrected Microanalyses were performed by Messrs G Cornali and W Egger (Leo) UV-Spectra (λ_{max}) were measured for solutions in 96% EtOH on a Perkin Elmer Lambda 5 spectrophotometer ^{1}H-NMR spectra (δ_H) and ^{13}C-NMR spectra (δ_C) (courtesy Mr N R. Andersen, Leo) were run on a Bruker AC-300 spectrometer for solutions in $CDCl_3$ using the solvent as internal standard ($CDCl_3$ = 76 8 ppm, residual $CHCl_3$ = 7 26 ppm these reference values correspond to internal Me_4Si = 0 0(0) ppm), 100 MHz ^{1}H-NMR spectra (δ_H) were run on a Jeol FX100 70 eV EI mass spectra (*m/z*) were obtained on a Hewlett-Packard HP5985 mass spectrometer fitted with a direct insertion probe

(1*S*,3*R*,5*E*,7*E*,22*ξ*)-1,3-Bis-(*t*-butyldimethylsilyloxy)-22-methylselenyl-24-oxa-9,10-secocholesta-5,7,10(19)-triene (13). A stirred solution of the seleno-acetal (**11**)[11] (435 mg, 0 58 mmol) in dry THF (5 mL) was cooled to -70 °C and treated dropwise over 5 min with *n*-BuLi (1 4 *M* in hexanes, 0.5 mL, 0 7 mmol) After 10 min, chloromethyl *i*-propyl ether[19] (freshly distilled, 95 mg, 0 87 mmol) was added, and after a further 30 min at -70 °C, the reaction solution allowed to warm to -20 °C over 30 min Ether (30 mL) was then added and the solution was extracted with 2% sodium hydrogen carbonate solution The organic phase was washed with brine, dried and concentrated *in vacuo* to give an oil Purification by chromatography (30 g silica gel, 2%, increasing to 5%, ether in petroleum ether as eluant) gave **13** (308 mg, 73%) as an oil that crystallised upon standing The product was chromatographically homogeneous (R_f *ca* 0 5, 5% ether in petroleum ether as eluant), although the NMR spectra showed that it consisted of a mixture of epimers at C-22 in a 3 2 ratio Recrystallisation from ether-methanol gave an analytical sample (needles, mp 110-112 °C), in which this ratio was increased to *ca* 2 1, Found C, 64 97, H, 10 04 Calcd for $C_{39}H_{72}O_3SeSi_2$ C, 64 69; H, 10 02%, λ_{max} 270 nm (ϵ 25500), δ_H (300 MHz) (*ca* 1$^{\#}$ 2*/2*.1$^{\#}$ integrated ratio of appropriate signals from the 22*R*$^{\#}$ and the 22*S** isomer respectively) 0 07 (br s, 12 H, SiCH_3's), 0 55* and 0 58$^{\#}$ [2 s (ratio *ca* 2* 1$^{\#}$), 3 H, 18-H_3], 0 87 and 0 90 (each: s, 9 H, SiC(CH_3)$_3$), *ca* 0 9$^{\#}$ (partially obscured, deduced from C-H correlation) and 1 03* [2 d (ratio *ca* 1$^{\#}$ 2*), J^{*} = 6 8 Hz, 3 H, 21-H_3], 1 15, 1.15 and 1 16 (overlapping d's, J = 6 Hz, 6 H, 26- and 27-H_3's), 2 02$^{\#}$ and 2 06* [2 s (ratio *ca* 1$^{\#}$ 2*), 3 H, SeCH_3], 2 30 (br d, J = 14 Hz, 1 H, 4β-*H*), 2 57 (dd, J = 5, 14 Hz, 1 H, 4α-*H*),

2 87 (b d, J = 12 Hz, 1 H, 9β-*H*), 2.97[#] and 3 15[*] [b t[#], J = 7.5 Hz, and ddd[*], J = 2, 5, 8 Hz (ratio *ca.* 1[#]:2[*]), 1 H, 22-*H*], 3 45-3.79 (m's, 23-H_2 and 25-*H*), 4 22 (m, 1 H, 3-*H*), 4.54 (m, 1 H, 1-*H*), 4.94 (br s, 1 H, 19*Z*-*H*), 4.99 (m, 1 H, 19*E*-*H*), 5 83 and 6.46 (each: d, J = 11.3 Hz, 1 H, 7-*H* and 6-*H*); δ_C (*ca.* 1[#]:2[*]/2[*]:1[#] ratio peak heights where appropriate) -5.1, -5 1, -5.0, -4 9 (SiCH$_3$'s), 4.1[#], 4.5[*] (SeCH$_3$), 11.5[*], 12.2[#] (*C*-18), 14.6[#], 15.6[*] (*C*-21), 17.9, 18.1 (SiCMe$_3$'s), 21 8, 21.9, 22 2 (*C*-26 and *C*-27), 22 0 (*C*-11), 23.3 (*C*-15), 25.6, 25.7 (SiCCH$_3$'s), 27 3[#], 27.5[*] (*C*-16), 28.7 (*C*-9), 36.4 (*C*-4), 36.4[#], 40.3[*] (*C*-20), 40.3 (*C*-12), 43.8 (*C*-2), 45.6[#], 46.0[*] (*C*-13), 46.7[*], 49 1[#] (*C*-22), 56 3 (*C*-17), 56.1[*], 56.2[#] (*C*-14), 67.0 (*C*-3), 69.1[*] (*C*-23), 70.0 (*C*-1), 70 8[#] (*C*-23), 71 5[#], 71 8[*] (*C*-25), 106.4 (*C*-19), 116.3[#], 116.4[*] (*C*-7), 121.5 (*C*-6), 135.2[#], 135.4[*] (*C*-5), 142.8[*], 143 1[#] (*C*-8) and 153 4 (*C*-10)

(1*S*,3*R*,5*E*,7*E*)-1,3-Bis-(*t*-butyldimethylsilyloxy)-24-oxa-9,10-secocholesta-5,7,10(19)-triene (6*R*)- and (6*S*)-sulphur dioxide adducts (17). Compound **13** (mixture of diastereoisomers, 360 mg, 0.50 mmol) was dissolved in ether (0 5 mL) and liquid SO_2 (10 mL) added The SO_2 was allowed to boil under reflux for 30 min and then the solvents were removed *in vacuo* to give a diastereoisomeric mixture of SO_2-adducts of the compounds **13**, showing two spots on TLC (R_f's *ca* 0 5 and 0.3, 40% ether in petroleum ether as eluant). [The ^{1}H-NMR spectra of the two components, separated in a *ca* 3:1 ratio from the product of a parallel experiment, showed the major (less polar) to be the 22-epimeric mixture of (6*S*)-sulphur dioxide adducts (6*S*)-**15** (18-H_3 singlets at δ 0.66[*] and 0.69[#]), while the minor component was the 22-epimeric mixture of (6*R*)-sulphur dioxide adducts (6*R*)-**15** (18-H_3 singlets at δ 0.56[*] and 0.59[#]) (*cf* ref. 11)] The product (in a pyrex flask) was dissolved in toluene (14 mL) and *n*-Bu$_3$SnH (0.87 g, 3 mmol) added. A nitrogen atmosphere was established, and the reaction flask was water cooled (20 °C) during illumination with radiation from a high pressure Hg lamp (type. Hanau TQ 718Z2) for 75 min The solution was then concentrated *in vacuo* to give a residue containing the title compounds, which was purified by chromatography (50 g silica gel, 30% ether in petroleum ether as eluant). First eluted was the major isomer (6*S*)-**17** (243 mg, 70%), needles from ether-methanol, mp 124-125 °C; Found: C, 65.63; H, 10 21; S, 4 50 Calcd for $C_{38}H_{70}O_5SSi_2$ C, 65 65, H, 10 15, S, 4 61%, δ_H (100 MHz) 0 06 (br s, 12 H, SiCH$_3$'s), 0 65 (s, 3 H, 18-H_3), 0 87 and 0 88 (each s, 9 H, SiC(CH$_3$)$_3$), 0.94 (d, J = 6 Hz, 3 H, 21-H_3), 1 13 (d, J = 6 Hz, 6 H, 26- and 27-H_3's), 3.2-4.1 (m's, including 3 53, hept, J = 6 Hz, 25-*H*, and 3.76, br ABq, J = 16 Hz, 19-H_2, 5 H, including also 23-H_2), 4 2 (m, 1 H, 3-*H*), 4 35 (m, 1 H, 1-*H*), 4.67 (m, 2 H, 6-*H* and 7-*H*) This was followed by the minor isomer (6*R*)-**17** (76 mg, 22%), obtained as a gum, δ_H (100 MHz) 0 07 (br s, 12 H, SiCH$_3$'s), 0 58 (s, 3 H, 18-H_3), 0 89 and 0 90 (each s, 9 H, SiC(CH$_3$)$_3$), 0.95 (d, J = 7 Hz, 3 H, 21-H_3), 1 15 (d, J = 6 Hz, 6 H, 26- and 27-H_3's), 3 25 - 4 1 (m's, including 3 53, hept, J = 6 Hz, 25-*H*, and 3 77, br ABq, J = 16 Hz, 19-H_2, 5 H, also including 23-H_2), 4.15 (m, 1 H, 3-*H*), 4 4 (m, 1 H, 1-*H*), 4 5-4 95 (2 br d, 2 H, J = 10 Hz, 6-*H* and 7-*H*)

(1*S*,3*R*,5*E*,7*E*)-1,3-Bis-(*t*-butyldimethylsilyloxy)-24-oxa-9,10-secocholesta-5,7,10(19)-triene (16). Compound [**17**, major (6*S*) isomer] (183 mg, 0 26 mmol) together with NaHCO$_3$ (0.3 g) was dissolved or suspended in 96% ethanol (10 mL), and the stirred mixture was heated under reflux for 90 min. After cooling, the reaction mixture was partitioned between EtOAc (30 mL) and water, and the EtOAc layer was washed with brine and dried Removal of the solvent *in vacuo* and purification by chromatography (15 g silica gel, 3% ether in petroleum ether as eluant) followed by crystallisation from ether-methanol gave **16** (140 mg, 84%) as needles, mp 76-77 °C, Found C, 72.39, H, 11.18 Calcd for $C_{38}H_{70}O_3SSi_2$· C, 72.32; H, 11 18%; λ_{max} 270 nm; δ_H (100 MHz) 0 07 (br s, 12 H, SiCH$_3$'s), 0 56 (s, 3 H, 18-H_3), 0.88 and 0 91 (each s, 9 H, SiC(CH$_3$)$_3$), 0 95 (d, J = 7 Hz, 3 H, 21-H_3), 1 15 (d, J = 6 Hz, 6 H, 26- and 27-H_3's), 3 2-3 7 (m, including 3 55, hept, J = 6 Hz, 25-*H*, 3 H, including 23-H_2), 4 22 (m, 1 H, 3-*H*), 4.54 (dd, J = 5, 9 Hz, 1 H, 1-*H*), 4 97 (m, 2 H, 19-H_2), 5 82 and 6 47 (each d, J = 11 Hz, 1 H, 7-*H* and 6-*H*)

(1*S*,3*R*,5*Z*,7*E*)-24-Oxa-9,10-secocholesta-5,7,10(19)-triene-1,3-diol (MC 1090, 8). A solution of compound (**16**) (49 mg, 0 08 mmol), anthracene (25 mg) and triethylamine (0 1 mL) in toluene (5 mL) in a pyrex flask was irradiated with light from a high pressure ultraviolet lamp, type TQ150Z2 (Hanau) at about 20°C for 1 h The reaction mixture was filtered, concentrated *in vacuo* and purified by chromatography (15 g silica gel, 2% ether in petroleum ether as eluant) to give (1*S*,3*R*,5*Z*,7*E*)-1,3-bis-(*t*-

butyldimethylsilyloxy)-24-oxa-9,10-secocholesta-5,7,10(19)-triene (**18**) (44 mg), obtained as a gum [λ_{max} 265 nm; δ_H (100 MHz) 0.07 (br s, 12 H, SiCH_3's), 0.54 (s, 3 H, 18-H_3), 0.88 (br s, 18 H, SiC(CH_3)$_3$'s), 0.95 (d, J = 6.5 Hz, 3 H, 21-H_3), 1 15 (d, J = 6 1 Hz, 6 H, 26- and 27-H_3's), 3.25-3.70 (m, including 3.55, hept, J = 6.1 Hz, 25-*H*, 3 H, including 23-H_2), 4.19 (m, 1 H, 3-*H*), 4.37 (m, 1 H, 1-*H*), 4 87 (d, J = 3 Hz, 1 H, 19*E*-*H*), 5.18 (m, 1 H, 19*Z*-*H*), 6.01 and 6.23 (each d, J = 11 Hz, 1 H, 7-*H* and 6-*H*).] A solution of the entire product (0.07 mmol) and *n*-Bu_4N^+ F^- (trihydrate, 0 1 g, 0.32 mmol) in THF (5 mL) was stirred at 60 °C for 50 min. The reaction mixture was partitioned between ethyl acetate (30 mL) and 2% sodium hydrogen carbonate solution (20 mL). The organic phase was washed with brine, dried and concentrated *in vacuo* to give an oil Purification by chromatography (15 g silica gel, 66% ethyl acetate in petroleum ether as eluant) gave the title compound **8** (21 mg, 67% from **16**), obtained as a gum; λ_{max} 264 nm (ϵ 17500); δ_H (300 MHz) 0.55 (s, 3 H, 18-H_3), 0.95 (d, J = 6 5 Hz, 3 H, 21-H_3), 1 15 (d, J = 6.0 Hz, 6 H, 26- and 27-H_3's), 2.31 (dd, J = 7, 13 Hz, 1 H, 4β-*H*), 2 59 (dd, J = 3, 13 Hz, 1 H, 4α-*H*), 2.82 (dd, J = 3, 11 Hz, 1 H, 9β-*H*), 3 33-3.60 (m, including 3.54, hept, J = 6.1 Hz, 25-*H*, 3 H, including 23-H_2), 4.23 (m, 1 H, 3-*H*), 4 43 (m, 1 H, 1-*H*), 5.00 (br s, 1 H, 19*E*-*H*), 5.33 (br s, 1 H, 19*Z*-*H*), 6.02 and 6.38 (each d, J = 11.3 Hz, 1 H, 7-*H* and 6-*H*), δ_C 12 0, 19.2, 22.1, 22.3, 22.3, 23.6, 27.6, 29.1, 33.7, 36.1, 40 5, 42 9, 45 3, 46.0, 56 4, 56.9, 66 3, 66.9, 70.9, 71 3, 111.8, 117.1, 125.0, 132.9, 143.2, and 147.6, *m/z* 402 (M^+, 6), 387 (0 5), 384 (6), 369 (1), 366 (3), 343 (1), 287 (2), 269 (3), 251 (5), 152 (30), and 134 (100%), HRMS (EI) calcd for $C_{26}H_{42}O_3$ 402 3134 (M^+); found: 402.3139.[9]

REFERENCES AND NOTES

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