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Synthesis of diacylglycerol analogues as potential second-messenger antagonists and inhibitors of protein kinase C

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ABSTRACT

A series of analogues of diacylglycerol has been prepared and tested as inhibitors of protein kinase C (PKC). The diketone analogues, 10-hydroxymethyl-8,13-eicosanedione (**24**), 10-acetoxymethyl-8,13-eicosanedione (**25**), and 10-methoxymethyl-8,13-eicosanedione (**26**) each inhibited PKC activated by 2-*O*-acetyl-1-*O*-oleoylglycerol. Compound **24** was the most effective inhibitor of the growth of MR4 and HT29 cells in culture, and **26** was more effective than **24** against HL60 cells.

INTRODUCTION

Diacylglycerol (DAG), which is mainly 2-*O*-arachidonyl-1-*O*-stearoyl-*sn*-glycerol[1] (**1**), results from the hydrolysis of membrane-bound inositol phospholipids by phospholipase C and is involved crucially in cell-signalling phenomena[2,3]. Together with *myo*-inositol 1,4,5-trisphosphate (**2**), which is liberated concomitantly, DAG acts as an intracellular second messenger. In the presence of phospholipid and calcium ions, DAG activates protein kinase C (PKC), which plays an important role in a range of biological processes, presumably through the phosphorylation of specific proteins. PKC is the probable site of action of the tumour-promoting phorbol esters[4,5] and its activation by DAG may also be important in stimulating cell growth. However, since the tumour-promoting phorbol esters are not metabolised readily[5], in contrast to DAG, they lead to uncontrolled growth and proliferation.

A consideration of these factors invites speculation on the possible control of cell proliferation by DAG antagonists, but structure–activity studies have led to relatively little success[6,7]. Daniel et al.[8] reported that PKC activity, stimulated by

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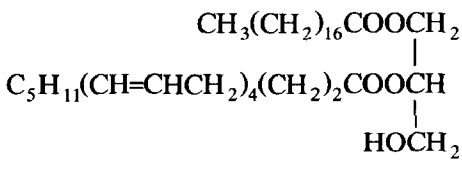
2-*O*-acetyl-1-*O*-oleoylglycerol, was inhibited by the addition of certain 2-*O*-acyl-1-*O*-alkylglycerols and 1,2-di-*O*-alkylglycerols, but Ganong et al.[9] found no significant inhibition of PKC activity, stimulated by 1,2-di-*O*-octanoylglycerol (**4**), by analogues that included compounds in which HO-3 in DAG was replaced variously be hydrogen, chlorine, thiol, methoxyl, hydroxymethyl, and 3-hydroxypropyl, O-1 or O-2 was replaced by NH, and the acyl groups were replaced by alkyl.

We now report the synthesis of several new types of DAG analogues and bioassays on some of these compounds. Some of this work was the subject of a preliminary communication[10].

RESULTS AND DISCUSSION

All chiral compounds in this study were prepared as racemates and all of the compounds, except the disulphonate **14**, the diurethane **15**, and the "inverse esters" **16** and **17**, are analogues of 1,2-di-*O*-octanoylglycerol (**4**) (a diacylglycerol that can easily penetrate cell membranes[11]) in that they possess a linear C_8 chain attached through oxygen, nitrogen, or carbon atoms to positions 1 and 2 of the glycerol moiety.



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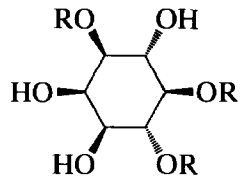
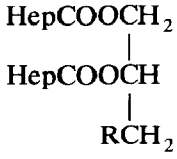
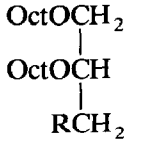


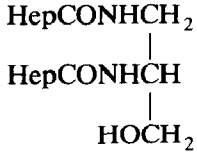
2 R = $P(O)(OH)_2$



3 R = OBn
4 R = OH
5 R = H
6 R = Cl
7 R = F
8 R = OMe



9 R = H
10 R = F
11 R = OMe
12 R = OH



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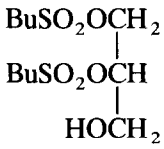
The 1,2-diacylglycerol analogues **5–8** were prepared by acylation of the appropriate diol with octanoyl chloride in pyridine. Propane-1,2-diol and 3-chloropropane-1,2-diol are commercially available. 3-Fluoro-1,2-propanediol was prepared[12] by treatment of commercially available epifluorohydrin with aqueous sulphuric acid and ethanol, and 1-*O*-methylglycerol was obtained by methylation of 1,2-*O*-

isopropylideneglycerol followed by acid hydrolysis. For reference purposes, 1,2-di-*O*-octanoylglycerol (**4**) was prepared by acylation of 1-*O*-benzylglycerol[13] (to give **3**) followed by catalytic hydrogenolysis.

The 1,2-di-*O*-alkyl analogues **9** and **11** were made by treatment of the parent diol with sodium hydride and octyl iodide in 1,2-dimethoxyethane. Octyl iodide and silver oxide were used to synthesise the fluoro analogue **10**, since 3-fluoro-1,2-propanediol would yield an epoxide on treatment with sodium hydride. Hydrogenolysis of 1-*O*-benzyl-2,3-di-*O*-octylglycerol, obtained by alkylation of 1-*O*-benzylglycerol, afforded 1,2-di-*O*-octylglycerol (**12**).

2,3-Dioctanamido-1-propanol (**13**) was prepared by acylation of the *O*-tetrahydropyranyl derivative of 2,3-diamino-1-propanol[14] with octanoyl chloride followed by acid hydrolysis. 1,2-Di-*O*-butanesulphonylglycerol (**14**) was synthesised by sulphonylation of 1-*O*-tritylglycerol[15] followed by treatment with acid. The 1,2-di-urethane (**15**) of glycerol was prepared by reacting 1-*O*-benzylglycerol with excess of butyl isocyanate followed by catalytic hydrogenolysis. The analogues **14** and **15** were prepared rather than the direct analogues of **4**, which would be derived from heptanesulphonyl chloride and hexyl isocyanate, respectively, because of the commercial availability of butanesulphonyl chloride and butyl isocyanate.



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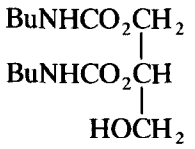

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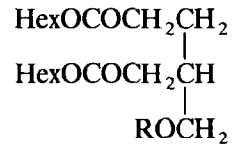
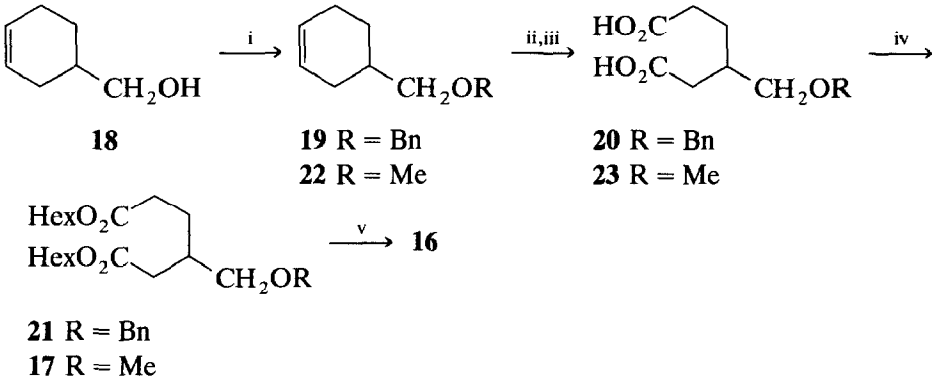


16 R = H
17 R = Me

The novel analogues **16** and **17** are based on the concept of "functional-group reversal". These compounds differ structurally from 1,2-di-*O*-octanoylglycerol (**4**) and the methyl ester **8**, respectively, in that the oxygen atoms and methylene groups α to the carbonyl group in both ester moieties have been interchanged. (±)-3-Cyclohexene-1-methanol (**18**, Scheme 1) was *O*-alkylated with benzyl bromide–sodium hydride and the resulting benzyl ether (**19**) was cleaved with ozone/oxygen in methanol. Oxidative work-up with hydrogen peroxide gave the diacid **20** which was esterified with 1-hexanol to afford the diester **21**, reductive hydrogenolysis of which gave dihexyl 3-(hydroxymethyl)hexanedioate (**16**). Similarly, the methyl ether **22**, readily obtained by *O*-methylation of **18**, could be oxidatively cleaved to the diacid **23** which was esterified to give dihexyl 3-(methoxymethyl)hexanedioate (**17**).

A ketone analogue (**24**) of a 1,2-di-*O*-acylglycerol, and the corresponding acetate **25** and methyl ether **26**, were prepared from *tert*-butyl nonanoate (**27**, Scheme 2), which was first self-coupled by sequential treatment with lithium di-isopropylamide and iodine to give the diester **28**. Reduction of **28** with lithium



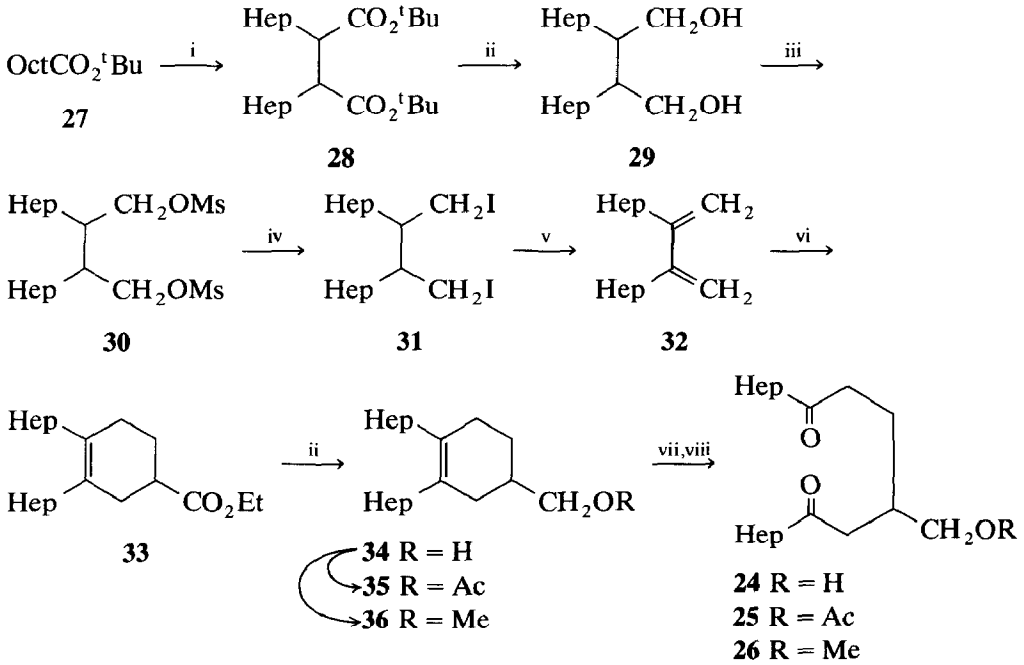
18 **19** R = Bn; **22** R = Me **20** R = Bn; **23** R = Me

21 R = Bn
17 R = Me

Reagents: (i) RHal/NaH/$(CH_2OMe)_2$; (ii) O_3/O_2-MeOH; (iii) H_2O_2/H_3O^+; (iv) $C_6H_{13}OH/H^+$; (v) H_2-Pd/MeOH

Scheme 1.

aluminium hydride afforded the diol **29** which, with methanesulphonyl chloride, gave the dimesylate **30**. Reaction of **30** with sodium iodide in butanone gave the di-iodide **31**. The conversion of **31** into the diene **32** was achieved with potassium *tert*-butoxide, and a Diels–Alder reaction of **32** with ethyl acrylate in toluene gave



27 **28** **29** **30** **31** **32** **33**

34 R = H; **35** R = Ac; **36** R = Me

24 R = H
25 R = Ac
26 R = Me

Reagents: (i) $LiN(iPr)_2/I_2$; (ii) $LiAlH_4/Et_2O$; (iii) MsCl/Et_3N/tetrahydrofuran/CH_2Cl_2; (iv) NaI/$CH_3COCH_2CH_3$; (v) ^{t}BuOK/BuOH; (vi) CH_2=$CHCO_2Et$/PhMe; (vii) O_3-O_2/EtOAc; (viii) Me_2S

Scheme 2.

4-ethoxycarbonyl-1,2-diheptylcyclohexene (**33**). Reduction of **33** with lithium aluminium hydride gave the alcohol **34**, which was converted by conventional procedures into the acetate **35** and the methyl ether **36**.

Ozonolysis of **34–36** was conducted in ethyl acetate, using dimethyl sulphide for reductive work-up, to give the diketone analogues **24–26**, respectively. Although the ether **26** was obtained analytically pure, all three compounds showed limited stability, especially in aqueous or alcoholic solutions, and characterisation of the alcohol **24** and the acetate **25** relied on spectroscopic data.

BIOLOGICAL RESULTS

In agreement with the results of Bell and co-workers[11,16], 1,2-di-*O*-octanoylglycerol (**4**) was found to activate protein kinase C (PKC). Of the other compounds, 2,3-dioctanamido-1-propanol (**13**) activated PKC, but not as effectively as 2-*O*-acetyl-1-*O*-oleoylglycerol (OAG). However, 10-hydroxymethyl-8,13-eicosanedione (**24**), 10-acetoxymethyl-8,13-eicosanedione (**25**), and 10-methoxymethyl-8,13-eicosanedione (**26**) each inhibited PKC activated by OAG, and the I_{50} values were ~ 0.01, ~ 0.01, and ~ 0.1 mM, respectively.

Of the inhibitory compounds, **24** was more effective than compound **26** at inhibiting growth of MR4 and HT29 cells in vitro, I_{80} concentrations being 0.05–0.1 mM for **24** and 0.1–1.0 mM for **26**. In earlier experiments with HL60 cells, **26** gave an I_{50} of 0.01 mM and **24** was rather less effective. Preliminary experiments suggest that **25** has a potency towards MR4 cells similar to that of **24**.

EXPERIMENTAL

Ether and 1,2-dimethoxyethane were dried over Na wire, and tetrahydrofuran was dried by distillation from Na in the presence of benzophenone as indicator. Pyridine was dried over KOH. Ethyl acetate, for use in ozonolysis reactions, was distilled from P_2O_5 and stored over molecular sieves (4A). Trityl chloride was recrystallised from toluene containing 1% (v/v) of acetyl chloride. All compounds described are racemic unless stated otherwise. Sodium hydride (60% dispersion in oil) was freed from oil by washing with light petroleum, and the weights noted refer to the washed and dried material. Organic solutions were dried over anhyd Na_2SO_4. Column chromatography was carried out on Silica Gel 60 (Merck). ^1H NMR spectra (60 MHz) were recorded on solutions in $CDCl_3$ (internal Me_4Si) with a JEOL PMX60si instrument, unless stated otherwise.

1-O-Benzyl-2,3-di-O-octanoylglycerol (**3**).—A solution of 1-*O*-benzylglycerol[13] (2.92 g, 16 mmol) in pyridine (12 mL) was treated with octanoyl chloride (5.74 g, 35 mmol) for 12 h, then poured into satd aq $NaHCO_3$ (100 mL). The mixture was extracted with CH_2Cl_2 (2 × 50 mL), and the combined extracts were washed successively with water, 2 M HCl, satd aq $NaHCO_3$, and water, dried, and concentrated. Column chromatography (light petroleum–EtOAc, 9:1) of the oily

residue yielded **3** (4.24 g, 61%); ν_{max}^{film} 1736 cm^{-1} (C=O). ^1H NMR data: δ 0.60–1.90 (complex, 26 H), 2.10–2.40 (m, 4 H, 2 CH_2COO), 3.55 (d, 2 H, J 4.8 Hz, CH_2OR), 4.10–4.30 (m, 2 H, CH_2OCO), 4.45 (s, 2 H, $PhCH_2$), 5.20 (quin, 1 H, J 4.8 Hz, CHOCO), 7.20 (bs, 5 H, Ph).

Anal. Calcd for $C_{26}H_{42}O_5$: C, 71.85; H, 9.7. Found: C, 71.7; H, 9.7.

1,2-Di-O-octanoylglycerol (**4**).—A solution of **3** (0.8 g, 1.84 mmol in MeOH (14 mL) and glacial acetic acid (2 mL) containing 5% Pd/C (0.15 g) was stirred under H_2 at room temperature for 19 h when the uptake of H_2 ceased. The solution was filtered and concentrated to give **4** (0.62 g, 98%); ν_{max}^{film} 3430 (OH) and 1735 cm^{-1} (C=O). ^1H NMR data: δ 0.70–1.90 (m, 26 H), 2.20–2.50 (m, 4 H, 2 CH_2CO), 3.45 (bs, 1 H, OH), 3.70 (d, 2 H, J 4.8 Hz, CH_2OH), 4.00–4.30 (m, 2 H, CH_2OCO), 5.10 (quin, 1 H, J 4.8 Hz, CHOCO).

Anal. Calcd for $C_{19}H_{36}O_5$: C, 66.2; H, 10.5. Found: C, 66.4; H, 10.6.

1,2-Dioctanoyloxypropane (1,2-propanediol dioctanoate, **5**).—Conventional acylation of 1,2-propanediol (2.28 g, 0.03 mol) with octanoyl chloride (10.76 g, 0.066 mol) in pyridine (10 mL), as described in the preparation of **3**, gave **5** (4.8 g, 49%), bp 108°/0.05 mmHg; ν_{max}^{film} 1738 cm^{-1} (C=O). ^1H NMR data: δ 0.80–1.90 (complex, 29 H), 2.10–2.40 (m, 4 H, 2 CH_2CO), 3.90–4.20 (m, 2 H, CH_2OCO), 5.10 (m, 1 H, CHOCO).

Anal. Calcd for $C_{19}H_{36}O_4$: C, 69.5; H, 11.1. Found: C, 69.4; H, 11.1.

3-Chloro-1,2-dioctanoyloxypropane (3-chloro-1,2-propanediol dioctanoate, **6**).—Acylation of 3-chloro-1,2-propanediol (6.63 g, 0.06 mol) in pyridine (20 mL) with octanoyl chloride (21.5 g, 0.13 mol), in the usual manner, gave, after chromatography (light petroleum–CH_2Cl_2, 3:2), liquid **6** (11 g, 51%); ν_{max}^{film} 1739 cm^{-1}. ^1H NMR data: δ 0.71–1.90 (complex, 26 H), 2.10–2.50 (m, 4 H, 2 CH_2CO), 3.60 (d, 2 H, J 4.8 Hz, CH_2Cl), 4.10–4.30 (m, 2 H, CH_2OCO), 5.20 (quin, 1 H, J 4.8 Hz, CHOCO).

Anal. Calcd for $C_{19}H_{35}ClO_4$: C, 62.9; H, 9.7; Cl, 9.8. Found; C, 63.2; H, 9.9; Cl, 9.7.

3-Fluoro-1,2-dioctanoyloxypropane (3-fluoro-1,2-propanediol dioctanoate, **7**).—3-Fluoro-1,2-propanediol (1 g, 10.6 mmol), prepared[12] from epifluorohydrin, was acylated with octanoyl chloride (3.8 g, 23.3 mmol) in the usual manner, to give **7** (2.21 g, 60%), bp 160°/0.02 mmHg; ν_{max}^{film} 1740 cm^{-1} (C=O). ^1H NMR data: δ 0.70–1.90 (complex, 26 H), 2.20–2.50 (m, 4 H, 2 CH_2CO), 4.40–5.50 (complex, 5 H, CH_2F, CHOCO, and CH_2OCO).

Anal. Calcd for $C_{19}H_{35}FO_4$: C, 65.9; H, 10.2. Found: C, 66.0; H, 10.4.

1-O-Methyl-2,3-di-O-octanoylglycerol (**8**).—Acylation of 1-*O*-methylglycerol (0.9 g, 8.5 mmol) with octanoyl chloride (3.10 g, 19.1 mmol) in pyridine (5 mL) and isolation of the product in the usual manner gave, after column chromatography (hexane–EtOAc, 3:2), the diester **8** (1.83 g, 60%); ν_{max}^{film} 1738 cm^{-1} (C=O). ^1H NMR data: δ (neat) 0.50–1.70 (complex, 26 H), 1.90–2.30 (m, 4 H, 2 CH_2CO), 3.15 (s, 3 H, OMe), 3.35 (d, 2 H, J 4.8 Hz, CH_2OMe), 3.70–4.30 (m, 2 H, CH_2OCO), 5.00 (m, 1 H, CHOCO).

Anal. Calcd for $C_{20}H_{38}O_5$: C, 67.0; H, 10.7. Found: C, 67.3; H, 10.4.

1,2-Dioctyloxypropane (**9**).—To a stirred solution of 1,2-propanediol (2.28 g, 0.03 mol) in 1,2-dimethoxyethane (75 mL) was added NaH (2.93 g, 0.12 mol) in portions followed, after 20 min, by octyl iodide (14.8 g, 0.062 mol). The mixture was stirred at room temperature for 18 h, heated under reflux for 1 h, cooled, diluted with EtOH (11 mL) and then water (3.5 mL), and concentrated. The residue was partitioned between CH_2Cl_2 and water, the aqueous layer was extracted several times with CH_2Cl_2, and the combined organic layers were washed with water, dried, and concentrated. The oily residue was distilled to yield **9** (1.10 g, 12%), bp 118°/0.05 mmHg. ^{1}H NMR data: δ 0.70–1.70 (complex, 33 H), 3.20–3.60 (complex, 7 H).

Anal. Calcd for $C_{19}H_{40}O_2$: C, 75.9; H, 13.4. Found: C, 75.9; H, 13.3.

3-Fluoro-1,2-dioctyloxypropane (**10**).—To a stirred solution of 3-fluoro-1,2-propanediol[12] (0.89 g, 9.4 mmol) in octyl iodide (11.2 g, 47 mmol) was added freshly prepared Ag_2O (5.47 g, 23.4 mmol) in portions over 5 h, and stirring was then continued at 50° for 1 h. Dichloromethane was added to the cooled solution, the mixture was filtered through kieselguhr, and concentrated. The oily residue was fractionally distilled and the crude product, bp 64–69°/0.08 mmHg, was redistilled to give **10** (0.17 g, 6%), bp 56–60°/0.01 mmHg. ^{1}H NMR data: δ 0.70–1.70 (complex, 30 H), 3.20–3.50 (complex, 4 H), 4.00–4.30 (m, 3 H), 4.80 (d, 2 H, $J_{H,F}$ 47.5 Hz, CH_2F).

Anal. Calcd for $C_{19}H_{39}FO_2$: C, 71.6; H, 12.3. Found: C, 71.2; H, 12.0.

1-O-Methyl-2,3-di-O-octylglycerol (**11**).—Alkylation of 1-*O*-methylglycerol (1.0 g, 9.43 mmol), using NaH (0.9 g, 37.5 mmol) and octyl iodide (8.65 g, 36 mmol), as described for preparation of **9**, with column chromatography (light petroleum–EtOAc, 19:1) of the product, gave **11** (0.32 g, 10%), isolated as an oil. ^{1}H NMR data: δ 0.70–1.70 (complex, 30 H), 3.20–3.60 (complex, 12 H).

Anal. Calcd for $C_{20}H_{42}O_3$: C, 72.7; H, 12.8. Found: C, 72.7; H, 12.9.

1,2-Di-O-octylglycerol (**12**).—1-*O*-Benzylglycerol[13] (5 g, 0.027 mol) was alkylated using NaH (2.64 g, 0.11 mol) and octyl iodide (13.3 g, 0.055 mol), as described for the preparation of **9**. Column chromatography (light petroleum–EtOAc, 9:1) of the product gave 1-*O*-benzyl-2,3-di-*O*-octylglycerol (1.62 g, 15%), isolated as an oil. ^{1}H NMR data: δ 0.70–1.70 (complex, 30 H), 3.20–3.60 (complex, 7 H), 4.45 (s, 2 H, PhCH_2), 7.20 (bs, 5 H, Ph).

A solution of the foregoing product (1.25 g, 3.07 mmol) in MeOH (10 mL) and glacial acetic acid (10 mL) was stirred under H_2 in the presence of 5% Pd/C (0.2 g). When the uptake of H_2 was complete (2 h), the suspension was filtered and concentrated. Column chromatography (light petroleum–EtOAc, 8:2) of the residue gave **12** (0.66 g, 68%); ν_{max}^{film} 3380 cm^{-1} (OH). ^{1}H NMR data: δ 0.70–1.70 (complex, 30 H), 2.90–3.70 (complex, 10 H).

Anal. Calcd for $C_{19}H_{40}O_3$: C, 72.1; H, 12.7. Found: C, 72.3; H, 13.0.

2,3-Dioctanamido-1-propanol (**13**).—2,3-Diamino-1-tetrahydropyran-2-yloxypropane[14] (6 g, 0.034 mol) was acylated with octanoyl chloride (12.2 g, 0.075 mol) in

pyridine (10 mL) in the conventional manner, and the product was isolated as a syrup (10.1 g, 70%).

A solution of the crude product (4.8 g, 0.011 mol) in EtOH (20 mL) containing *p*-toluenesulphonic acid (0.1 g, 5 mmol) was stored at room temperature for 20 min, then neutralised with satd aq $NaHCO_3$, and concentrated. Column chromatography (EtOAc) of the residue gave, first, 2,3-dioctanamido-1-propanol octanoate. The second component eluted was crystallised from EtOAc–hexane to give **13** (1.44 g, 37%), mp 95–96°; ν_{max}^{Nujol} 3300 (OH), 1634, 1555 cm^{-1} (NHCO). ^1H NMR data: δ 0.70–1.90 (complex, 26 H), 2.00–2.40 (m, 4 H, CH_2CO), 3.20–4.10 (m, 5 H, CH_2N, CHN, and CH_2O), 4.40–4.80 (bs, 1 H, OH), 6.60–7.60 (bs, 2 H, 2 NH).

Anal. Calcd for $C_{19}H_{38}N_2O_3$: C, 66.6; H, 11.2; N, 8.2. Found: C, 66.9; H, 11.4; N, 8.1.

2,3-Dioctanamido-1-propanol octanoate, on treatment with methanolic NaOMe, was converted into **13**.

1,2-Di-O-butanesulphonylglycerol (**14**).—A solution of 1-*O*-tritylglycerol[15] (1.34 g, 4 mmol) in pyridine (10 mL) was treated with butanesulphonyl chloride (1.38 g, 8.82 mmol) for 12 h. Column chromatography (light petroleum–EtOAc, 7:3) of the crude product, isolated in the usual manner, gave 2,3-di-*O*-butanesulphonyl-1-*O*-tritylglycerol (1.63 g), isolated as a clear oil. A portion (1.51 g, 2.63 mmol) of this disulphonate was detritylated with formic acid in ether[17]. Column chromatography (light petroleum–EtOAc, 6:4) of the product gave **14** (0.41 g, 47%), isolated as a viscous oil; ν_{max}^{film} 3450 (OH), 1350, 1164 cm^{-1} (SO_2O). ^1H NMR data: δ 0.80–2.20 (complex, 14 H), 2.40–2.90 (bs, 1 H, OH), 3.10–3.40 (m, 4 H, 2 CH_2SO_2O), 3.90 (d, 2 H, *J* 5 Hz, CH_2OH), 4.45 (d, 2 H, CH_2OSO_2), 4.90 (quin, 1 H, $CHOSO_2$).

Anal. Calcd for $C_{11}H_{24}O_7S_2$: C, 39.7; H, 7.3; S, 19.3. Found: C, 39.8; H, 7.3; S, 18.9.

1,2-Di-O-butylcarbamoylglycerol (**15**).—1-*O*-Benzylglycerol[13] (1.55 g, 8.52 mmol) was added dropwise to stirred butyl isocyanate (17.3 g, 0.175 mol) at −78° and, after 5 min, stirring was continued at 60° for 10 min, then the excess of isocyanate was evaporated. Column chromatography (light petroleum–EtOAc, 6:4) of the residue gave 1-*O*-benzyl-2,3-di-*O*-butylcarbamoylglycerol (0.62 g, 19%), isolated as an oil; ν_{max}^{film} 3340 (NH), 1698, 1530 cm^{-1} (CO). ^1H NMR data: δ 0.80–1.90 (complex, 14 H), 3.00–3.30 (m, 4 H, 2 CH_2N), 3.60 (d, 2 H, *J* 4.8 Hz, CH_2OR), 4.25 (d, 2 H, *J* 4.8 Hz, CH_2OCO), 4.55 (s, 2 H, $PhCH_2$), 4.75 (bs, 2 H, 2 NH), 5.10 (quin, 1 H, CHOCO), 7.30 (bs, 5 H, Ph).

Anal. Calcd for $C_{20}H_{32}N_2O_5$: C, 63.1; H, 8.5; N, 7.4. Found: C, 62.6; H, 8.5; N, 6.9.

A solution of the foregoing benzyl ether (0.42 g, 1.1 mmol) in MeOH (10 mL) was stirred under a slight overpressure of H_2 in the presence of 5% Pd/C (0.1 g) for 18 h, when the uptake of H_2 was complete. The solution was filtered and concentrated to give an oil which crystallised to afford **15** (0.27 g, 84%), mp 59–61°; ν_{max}^{Nujol} 3330 (OH, NH), 1698 and 1534 cm^{-1} (CO). ^1H NMR data: δ

0.70–1.60 (complex, 14 H), 2.90–3.30 (m, 4 H, 2 CH_2N), 3.50–3.80 (m, 3 H, CH_2OH), 4.20 (d, 2 H, *J* 5 Hz, CH_2OCO), 4.85 (quin, 1 H, *J* 5 Hz, CHOCO), 5.30 (bs, 2 H, 2 NH).

Anal. Calcd for $C_{13}H_{26}N_2O_5$: C, 53.7; H, 9.0; N, 9.6. Found: C, 53.5; H, 9.1; N, 9.3.

Dihexyl 3-(hydroxymethyl)hexanedioate (**16**).—3-Cyclohexene-1-methanol (**18**; 6.73 g, 0.06 mol) was treated with NaH (2.88 g, 0.12 mol) and benzyl bromide (11.3 g, 0.066 mol) in 1,2-dimethoxyethane (90 mL), in the usual manner, to give 4-(benzyloxymethyl)cyclohex-1-ene (**19**; 10.7 g, 88%), bp 86–88°/0.06 mmHg. ^{1}H NMR data: δ 1.70–2.20 (complex, 7 H), 3.40 (d, 2 H, *J* 5.3 Hz, CHCH_2O), 4.50 (s, 2 H, PhCH_2), 5.60 (m, 2 H, CH=CH), 7.30 (s, 5 H, Ph).

Anal. Calcd for $C_{14}H_{18}O$: C, 83.1; H, 9.0. Found: C, 83.3; H, 9.2.

An O_3–O_2 mixture was passed through a solution of a portion (5 g, 24.7 mmol) of **19** in MeOH (40 mL) at −78° until the effluent gas liberated iodine from acidified aq KI. The solution was then flushed with N_2 and the solvent was evaporated at room temperature. The residue was dissolved in aq 90% formic acid (20 mL), and aq 30% H_2O_2 was added. The mixture was warmed to 40° for 30 min, then heated under reflux for 30 min, and concentrated. A solution of the residue in ether was filtered and concentrated to give the crude diacid **20** as a viscous oil (5.85 g).

A mixture of crude **20** (2 g), hexanol (2.5 g), concd H_2SO_4 (0.03 mL), and toluene (15 mL) was heated under reflux for 18 h in a Dean–Stark apparatus to remove water. Ether (10 mL) was added, the solution was washed with aq 10% Na_2CO_3 (twice) and aq NaCl, then dried, and concentrated. Column chromatography (light petroleum–EtOAc, 9:1) of the residue afforded dihexyl 3-(benzyloxymethyl)hexanedioate (**21**; 2 g, 61%), isolated as an oil; ν_{max}^{film} 1732 cm^{-1} (CO). ^{1}H NMR data: δ 0.70–1.90 (complex, 25 H), 2.10–2.50 (m, 4 H, 2 CH_2CO), 3.35 (d, 2 H, *J* 4.8 Hz, CHCH_2O), 4.00 (t, 4 H, 2 $COOCH_2$), 4.40 (s, 2 H, PhCH_2), 7.20 (s, 5 H, Ph).

Anal. Calcd for $C_{26}H_{42}O_5$: C, 71.8; H, 9.7. Found: C, 71.6; H, 9.9.

A solution of the di-ester **21** (1.34 g, 3.03 mmol) in MeOH was hydrogenolysed at atmospheric pressure over 5% Pd/C (0.45 g). After the uptake of H_2 was complete (18 h), the solution was filtered and concentrated. Column chromatography (ether–hexane, 8:2) of the residue gave **16** (0.64 g, 60%), isolated as a liquid; ν_{max}^{film} 3450 (OH), 1734 (CO) cm^{-1}. ^{1}H NMR data: δ 0.70–1.90 (complex, 25 H), 2.20–2.50 (m, 4 H, 2 CH_2CO), 3.60 (complex, 3 H, CH_2OH), 4.05 (t, 4 H, *J* 5.8 Hz, 2 $COOCH_2$).

Anal. Calcd for $C_{19}H_{36}O_5$: C, 66.2; H, 10.5. Found: C, 66.3; H, 10.6.

Dihexyl 3-(methoxmethyl)hexanedioate (**17**).—3-Cyclohexene-1-methanol (**18**; 6.73 g, 0.06 mol) was treated with NaH (2.88 g, 0.12 mol) and MeI (9.2 g, 0.066 mol) in 1,2-dimethoxyethane (90 mL), in the usual manner, to give 4-(methoxymethyl)cyclohex-1-ene (**22**; 3.05 g, 40%), bp 42°/8 mmHg. ^{1}H NMR data: δ (neat) 0.80–2.40 (complex, 7 H), 3.00–3.40 (m, 2 H, MeOCH_2), 3.20 (s, 3 H, MeO), 5.50 (b, 2 H, CH=CH).

Anal. Calcd for $C_8H_{14}O$: C, 76.1; H, 11.2. Found: C, 76.1; H, 11.2.

Treatment of **22** with O_3–O_2 and oxidative work-up with H_2O_2, as described in the preparation of **20**, gave the crude di-acid **23** (2.38 g), which was esterified with hexanol as described in the preparation of **21**. Column chromatography (light petroleum–EtOAc, 9:1) of the product gave **17** (1.49 g, 33%), isolated as an oil; ν_{max}^{film} 1736 cm^{-1} (CO). ^1H NMR data: δ 0.70–1.90 (complex, 25 H), 2.20–2.50 (m, 4 H, 2 CH_2CO), 3.20–3.30 (m, 2 H, $MeOCH_2$), 3.32 (s, 3 H, MeO) 4.00 (t, 4 H, *J* 6.5 Hz, 2 $COOCH_2$).

Anal. Calcd for $C_{20}H_{38}O_5$: C, 67.0; H, 10.7. Found: C, 66.9; H, 10.7.

*Di-*tert*-butyl 2,3-diheptylsuccinate* (**28**).—*tert*-Butyl nonanoate (**27**, 25 g, 0.12 mol), prepared by conventional acylation of *tert*-butyl alcohol with nonanoyl chloride in pyridine, was coupled[18] by sequential treatment of a solution in tetrahydrofuran with a solution of lithium di-isopropylamide (0.12 mol, prepared by reacting equimolar amounts of di-isopropylamine in tetrahydrofuran with butyllithium in hexane) and I_2 (0.06 mol). A small amount of starting material was distilled from the crude product (26.7 g) at 105°/0.02 mmHg. A solution of the residue in light petroleum was decolourised with charcoal, then concentrated to a pale-yellow oil (24.2 g) that was sufficiently pure (^1H NMR spectroscopy) for use in the next stage. A portion was distilled to give an analytical sample of **28**, bp 170°/0.01 mmHg; ν_{max}^{film} 1722 cm^{-1} (CO). ^1H NMR data: δ 0.70–2.00 (complex, 48 H, 2 Hep and 2 ^{t}Bu), 2.50 (b, 2 H, 2 $CHCO_2R$).

Anal. Calcd for $C_{26}H_{50}O_4$: C, 73.2; H, 11.8. Found: C, 73.5; H, 11.9.

2,3-Diheptyl-1,4-dimethanesulphonyoxybutane (2,3-diheptyl-1,4-butanediol dimethanesulphonate, **30**).—Compound **28** (22.7 g, 0.053 mol) was added slowly to a solution of $LiAlH_4$ (4.1 g, 0.11 mol) in ether (120 mL) at 0°, and the mixture was stirred for 1 h, then heated under reflux for 1 h. Aqueous 15% NaOH (4 mL) and then water (17 mL) were added with stirring. The mixture was filtered, dried, and concentrated to afford crude syrupy 2,3-diheptyl-1,4-butanediol (**29**, 14.8 g).

Methanesulphonyl chloride (4.95 g, 0.043 mol) was added to an ice-cold, stirred solution of the crude 1,4-diol (4 g, 0.013 mol) and triethylamine (5.15 g, 0.051 mol) in tetrahydrofuran (40 mL) and CH_2Cl_2 (100 mL). After 1 h, the solution was poured onto ice (130 g) and extracted with ether, and the extract was washed with aq NaCl, dried, and concentrated. The residue was recrystallised from hexane to give **30** (5.14 g, 86%), mp 53–55°. ^1H NMR data: δ 0.70–2.10 (complex, 32 H, 2 Hep and 2 CH), 3.00 (s, 6 H, 2 Ms), 4.20 (d, 4 H, *J* 4.8 Hz, 2 CH_2OMs).

Anal. Calcd for $C_{20}H_{42}O_6S_2$: C, 54.3; H, 9.6; S, 14.5. Found: C, 54.4; H, 9.5; S, 14.5.

2,3-Diheptyl-1,3-butadiene (**32**).—A solution of **30** (11.2 g, 0.024 mol) and NaI (18 g, 0.12 mol) in butanone (400 mL) was heated under reflux for 4 h, then cooled, filtered, and concentrated. A solution of the residue in ether was washed twice with aq sodium thiosulphate, dried, and concentrated to give the crude oily di-iodide **31** (11.7 g).

A solution of the crude di-iodide (2.65 g, 5 mmol) and potassium *tert*-butoxide

(1.7 g, 15.2 mmol) in butyl alcohol was heated under reflux for 18 h, then poured into water (200 mL), and extracted with light petroleum (3 × 30 mL). The combined extracts were washed with water, dried, and concentrated to leave a viscous oil which was distilled to give **32** (0.37 g, 30%), bp 121°/0.8 mmHg; lit.[19] bp 95–105°/0.015 mmHg. ^{1}H NMR data: δ (neat) 0.60–1.80 (complex, 26 H, 2 Hex), 1.90–2.30 (m, 4 H, 2 CH_2C=C), 4.75 and 4.90 (2 bs, each 2 H, 2 H_2C=C).

Anal. Calcd for $C_{18}H_{34}$: C, 86.3; H, 13.7. Found: C, 86.2; H, 13.7.

4-Ethoxycarbonyl-1,2-diheptylcyclohexene (**33**).—A solution of **32** (2.52 g, 10.1 mmol) and ethyl acrylate (1.22 g, 12.2 mmol) in toluene (15 mL) was heated under reflux for 5 days, then concentrated. Column chromatography (light petroleum–EtOAc, 19:1) of the residue yielded **33** (3.12 g, 88%), isolated as an oil; ν_{max}^{film} 1740 cm^{-1} (CO). ^{1}H NMR data: δ 0.60–1.60 (complex, 31 H), 1.70–2.50 (complex, 9 H, 4 CH_2C=C and -$CHCO_2R$), 4.10 (q, 2 H, *J* 7.2 Hz, MeCH_2O).

Anal. Calcd for $C_{23}H_{42}O_2$: C, 78.8; H, 12.1. Found: C, 78.8; H, 12.3.

10-Hydroxymethyl-8,13-eicosanedione (**24**).—To a stirred suspension of $LiAlH_4$ (0.57 g, 15.4 mmol) in dry ether (50 mL) at 0° under nitrogen was added dropwise a solution of **33** (3.1 g, 8.85 mmol) in ether (20 mL). The mixture was heated under reflux for 1 h, then cooled in an ice bath. Aqueous 15% NaOH (0.6 mL) was added and then water (2.3 mL), and the mixture was stirred for 30 min and filtered. The insoluble material was washed with ether, and the combined filtrates and washings were dried, then concentrated to give crude oily 1,2-diheptyl-4-(hydroxymethyl)cyclohexene (**34**, 2.46 g), ν_{max}^{film} 3300 cm^{-1} (OH).

An O_3–O_2 mixture was passed through a solution of crude **34** (0.97 g, 3.01 mmol) in EtOAc (30 mL) at −78°, until the outlet gas liberated I_2 from aq acidic KI. The solution was then flushed with N_2, and Me_2S (0.4 mL) was added to the stirred solution, which was maintained at −70° for 1 h, then at 20° for 1 h. The solvent was evaporated, the residue was triturated with hexane, and the extract was filtered and concentrated. Column chromatography (EtOAc) of the residue afforded **24** (0.83 g, 79%), isolated as a viscous oil; ν_{max}^{film} 3380 (OH), 1712 cm^{-1} (CO). ^{1}H NMR data: δ 0.70–2.60 (complex, 38 H), 3.40–3.60 (m, 2 H, CH_2OH). Mass spectrum: *m/z* 340.2977 ($C_{21}H_{40}O_3$).

The product could not be distilled without decomposition.

10-Acetoxymethyl-8,13-eicosanedione (**25**).—The alcohol **34** (2 g, 6.48 mmol) was treated with acetic anhydride (3 mL) in pyridine (8 mL) in the usual manner. Column chromatography (light petroleum–EtOAc, 9:1) of the product gave 4-acetoxymethyl-1,2-diheptylcyclohexene (**35**; 1.77 g, 80%), isolated as an oil; ν_{max}^{film} 1732 cm^{-1} (CO). ^{1}H NMR data: δ 0.80–2.40 (complex, 40 H), 3.20–3.50 (m, 2 H, CH_2OAc).

Anal. Calcd for $C_{23}H_{42}O_2$: C, 78.8; H, 12.1. Found: C, 78.8; H, 12.0.

An O_3–O_2 mixture was passed through a solution of **35** (2.54 g, 7.25 mmol) in EtOAc (75 mL), as described for **34**. Column chromatography (light petroleum–EtOAc, 8:2) of the product gave **25** (2.22 g, 80%), isolated as an oil; ν_{max}^{film} 1745, 1720 cm^{-1} (CO). ^{1}H NMR data: δ 0.70–1.90 (complex, 29 H), 2.00 (s, 3 H, Ac),

1.20–1.60 (m, 8 H, 4 CH_2CO), 3.90–4.10 (m, 2 H, CH_2COOMe). Mass spectrum: m/z 382.3083 ($C_{23}H_{42}O_4$).

10-Methoxymethyl-8,13-eicosanedione (**26**).—The alcohol **34** (3 g, 9.72 mmol) was treated with NaH (0.47 g, 19.4 mmol) and MeI (1.5 g, 10.5 mmol) in 1,2-dimethoxyethane (30 mL) in the usual manner. Column chromatography (light petroleum–EtOAc, 19:1) of the product gave **36**, isolated as a crude oil (2.93 g).

A solution of crude **36** (0.15 g) in EtOAc (6 mL) was treated with O_3–O_2, as described for the preparation of **24**, to give a viscous oil (0.13 g) that crystallised on storage to a solid, mp 27–30°, which was distilled to afford **26**, bp 170°/0.01 mmHg; ν_{max}^{film} 1710 cm^{-1} (CO). ^1H NMR data: δ 0.70–1.80 (complex, 29 H), 2.20–2.50 (m, 8 H, 4 CH_2CO), 3.20–3.30 (m, 5 H, CH_2OMe). Mass spectrum: m/z 354.3134 ($C_{23}H_{42}O_3$).

Anal. Calcd for $C_{23}H_{42}O_3$: C, 74.5; H, 11.9. Found: C, 74.3; H, 11.7.

Biological assays.—(a) Cell culture. The cell lines used were HL60 (derived from a human leukemia), HT29 (derived from a human colo-rectal tumour), and MR4 (a murine cell line possessing an active human oncogene, ras T24). The cells were grown in 25-cm tissue culture flasks in Dulbeccos minimal essential medium containing 10% of foetal calf serum (FCS), at 37° in a flow-CO_2 incubator until they reached a density of 1.5×10^4–2×10^5 cells/mL.

Each test compound was dissolved in EtOH and added at the required final concentration in 2% FCS to the cells in 96-well plates. After 72 h exposure, the cytoxic effects were estimated by the FRAME toxicity test[20]. Cell growth was measured by Kenacid Blue R binding[21].

(b) Protein kinase C (PKC) assay. Rat brain was homogenised in 20 mM Tris-HCl (pH 7.5), 10 mM EGTA, 2 mM EDTA, 2 MM PMSF, and 10 mM dithiothreitol. After centrifugation at 100 000g for 60 min, the supernatant solution was passed through a column of Sephadex G25 equilibrated with the above buffer without dithiothreitol. The protein-containing fraction was collected and used without further purification.

PKC activity was measured by incorporation of ^{32}P from γ-^{32}P-ATP into histone IIIS. Each assay contained, in a volume of 0.23 mL, 20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM $CaCl_2$, 200 μg of histone IIIS, 6 μg of phosphatidylserine (PS), and 2.8 μg of 2-*O*-acetyl-1-*O*-oleoylglycerol (OAG). To this solution was added 20 μL of the enzyme solution, and the reaction was started by the addition of 20 μL of 250 μM ATP containing 50 000 dpm of γ-^{32}P-ATP. After storage for 7 min at 30°, the reaction was quenched by the addition of 50-μL aliquots of the reaction mixture to 1 mL of aq 25% trichloroacetic acid at 0°. Acid-precipitable material was collected on 0.45-μm membrane filters, washed with 4 mL of aq 25% trichloroacetic acid, and subjected to scintillation counting.

The DAG analogues were tested for activation (each analogue was substituted for OAG in the above assay) and inhibition of PKC (each analogue was added to the above reaction mixture in the presence of PS and OAG). In the latter assay, each analogue was added in a small volume of EtOH, with an appropriate volume of EtOH being added to the control.

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