Experimental Section

3: A solution of **1** (264 mg, 0.24 mmol) in toluene (5 mL) was stirred for 2 h at 70 °C. During the reaction the color of the solution changed from yellow to orange. Gas-chromatographic analysis of the solution showed quantitative formation of benzene. The $^{31}P\{^1H\}$ NMR spectrum of the mixture after the reaction showed only the signal for **3**. Addition of pentane caused separation of **3** as yellow-orange crystals, which were collected by filtration and recrystallized from toluene/pentane (71 mg, 29% yield). The similar reaction of **2** gave **4** in 51% yield.

The kinetic measurements of the conversion were carried out in a thermostatted (70 °C) NMR probe by following the changes in the intensities of the ^1H NMR signal for the hydrido ligands of **1**. Dioxane was used as an internal standard.

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[7] **2**: ^1H NMR (C_6D_6, TMS): $\delta = 7.59$ (dd, $^3J(H,H) = 9$, $^3J(H,F) = 2$ Hz, 12H; aromatic H), 6.90 (t, $^3J(H,H) = {}^3J(H,F) = 9$ Hz, 12H; aromatic H), 1.54 (m, $^3J(P,H) = 7$ Hz, 6H; P–CH), 0.82 (br, 36H; CH_3), −12.55 (tt, $^1J(Rh,H) = 58$, $^2J(P,H) = 25$ Hz, 1H; Rh-H-Rh), −16.07 (AA′ part of an AA′MM′XX′ pattern, 2H; Rh–H); $^{31}P\{^1H\}$ NMR (C_6D_6, 85% H_3PO_4): $\delta = 54.2$ (AA′ part of an AA′XX′ pattern).

[8] Crystallographic data for **4**: $C_{48}H_{64}ClF_5P_2Rh_2Si_2$, $M_r = 1095.41$, monoclinic, space group $P2_1/c$ (no. 14), $a = 18.146(10)$, $b = 12.806(8)$, $c = 21.512(9)$ Å, $\beta = 90.39(3)°$, $V = 4998$ Å^3, $Z = 4$, $\mu = 8.74$ cm^{-1}, $F(000) = 2248$, $\rho_{calcd} = 1.455$ g cm^{-3}. The final R factor was 0.075 ($Rw = 0.059$) for 7999 reflections with $I > 3\sigma(I)$. Data collection on a RIGAKU-AFC5R diffractometer at 298 K with graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71073$ Å). The structure was solved with the program teXsan. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-100766. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: int. code + (49) 1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

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[10] **3**: ^1H NMR (C_6D_6, TMS): $\delta = 6.9 - 8.2$ (m, 25H; aromatic H), 1.18 (m, $^3J(P,H) = 7$ Hz, 6H; P–CH), 0.95 (m, 36H; CH_3), −17.40 (AA′ part of an AA′MM′XX′ pattern, 2H; Rh–H); $^{31}P\{^1H\}$ NMR (C_6D_6, 85% H_3PO_4): $\delta = 57.4$ (AA′ part of an AA′XX′ pattern); $^{29}Si\{^1H\}$ NMR (C_6D_6, TMS): $\delta = 157.8$ (t, $^2J(P,Si) = 50$ Hz), 28.7 (tt, $^2J(P,Si) = 50$, $^1J(Rh,Si) = 8$ Hz). **4**: ^1H NMR (C_6D_6, TMS): $\delta = 6.76 - 7.88$ (m, 20H; aromatic H), 1.12 (m, 6H; P–CH), 0.86 (m, 36H; CH_3), −17.60 (AA′ part of an AA′MM′XX′ pattern, 2H; Rh–H); $^{31}P\{^1H\}$ NMR (C_6D_6, 85% H_3PO_4): $\delta = 58.2$ (AA′ part of an AA′XX′ pattern).

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A Glucose-Containing Ether Lipid (Glc-PAF) as an Antiproliferative Analogue of the Platelet-Activating Factor**

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It has long been known that the platelet-activating factor (PAF) is a biologically highly active phosphoglyceride,[1] and various PAF analogues have been reported as inhibitors of proliferation.[2] However, since synthetic phospholipids are strongly cytotoxic, their therapeutic use has hitherto been restricted to topical applications.[3] We reported earlier the synthesis of a new type of glyceroglucolecithin (Glc-PC)[4] which displayed antiproliferative activity without cytotoxicity at concentrations below 10 μmol L^{-1}. We now present the glycoside of the ether analogue [1-*O*-octadecyl-2-*O*-α-D-glucopyranosyl-*sn*-glycero(3)]phosphorylcholine (Glc-PAF, **1**), which is formally derived from the PAF by exchanging the 2-acyl group for a glucose molecule.

The glycerol skeleton is provided by the starting material (*S*)-isopropylidene glycerol (**2**),[5] whose hydroxyl group is protected as the allyl ether functionality in **3** for introduction of the end groups (Scheme 1). The primary hydroxyl group of the diol **4** released upon acid hydrolysis cannot react directly to form an ether, and it is therefore first converted into a terminal benzoate ester group.[6] Compound **5** undergoes

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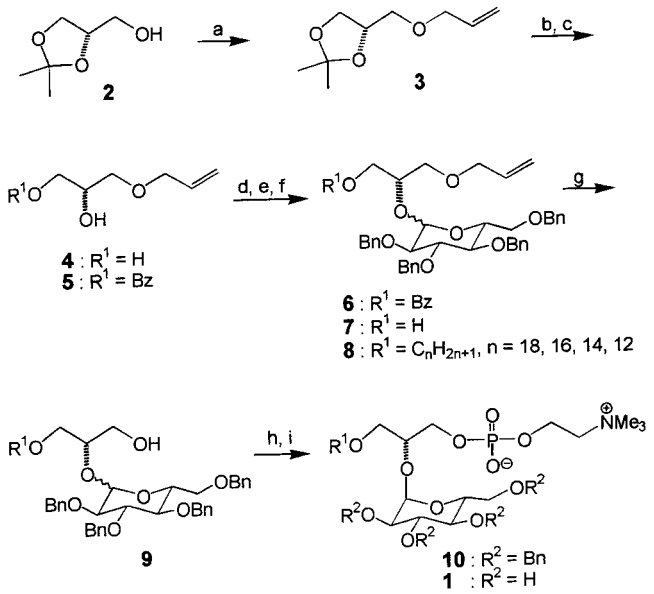

Scheme 1. Synthesis of **1**. a) NaH, allyl bromide, THF, 0 °C (98 %); b) AcOH/H_2O (60/40) (73 %); c) BzCl, DMAP, py, 0 °C (76 %); d) 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl fluoride, $AgClO_4$, $SnCl_2$, 4-Å molecular sieves, Et_2O, −15 °C (81 %); e) KOH/MeOH (30 %), MeOH, (91 %); f) NaH, RBr, THF, 80 °C (83–98 %); g) Pd/C (10 %), *p*-toluenesulfonic acid, MeOH/*i*PrOH/H_2O (4/4/1), 80 °C (70–94 %); h) 1. $POCl_3$, Et_3N, $CHCl_3$, 0 °C; 2. choline tosylate, py, 0 °C (47–74 %); i) H_2 (2 atm), Pd/C (5 %), MeOH (71–97 %); Bn = benzyl, Bz = benzoyl, DMAP = 4-(dimethylamino)pyridine, py = pyridine, R = C_nH_{2n+1}, n = 18, 16, 14, 12.

reaction with 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl fluoride[8] at the secondary alcohol functionality to provide glycoside **6**[9] according to Mukaiyama et al.[7] After saponification of the benzoate, the resulting compound **7** is converted into the ether with the appropriate alkyl bromide. Since the activity of choline-containing phospholipid analogues strongly depends on the length of the alkyl chain,[10] the chain length of the ether introduced at this stage (C_{18}, C_{16}, C_{14}, and C_{12}) corresponds to that of a biologically relevant derivative. Formation of the ether is highly temperature-dependent, and complete conversion into **8** does not occur until 80 °C.

Before introducing the final glycerol substituent (i.e. the phosphate), the original allyl ether must be cleaved by hydrogenolysis.[11] Alcohol **9** is then esterified with phosphoryl chloride, and the resulting dichloroester is immediately converted into the desired phosphocholine derivative **10** with choline tosylate.[12,13] Cleavage of the benzyl protecting groups presented considerable problems in the synthesis of the ester analogue Glc-PC. The ethers are, however, much more reactive, and the products are obtained in good yields after only short periods of hydrogenation. Starting from **2**, gram quantities of **1** were synthesized in nine steps with a total yield of 25 %.[14]

The biological activity of **1** was determined in a serum-free cell-culture system of human keratinocytes (HaCaT cells) by measuring three typical cell parameters. First, it was shown that **1** (n = 18) is nontoxic at concentrations below 4 μmol L^{-1} (Figure 1 a). Higher concentrations cause marked cell damage; the median lethal dose (LD_{50}) was 9 μmol L^{-1}. The growth-inhibitory properties of **1** were then investigated. As

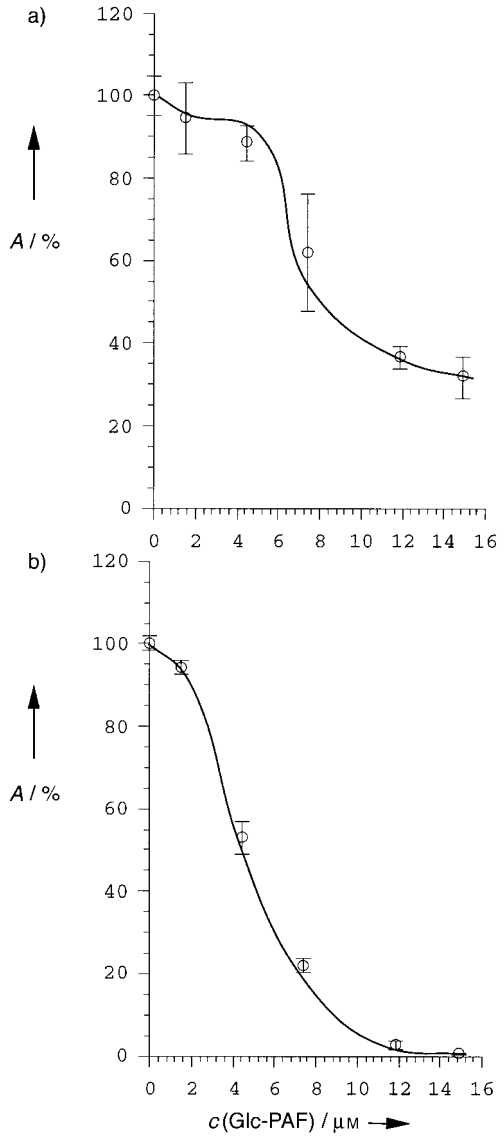

Figure 1. Influence of **1** (n = 18) on the viability (a) and proliferation (b) of HaCaT cells. Both are expressed as a percentage A of the value measured for the control experiment; c = concentration. Four experiments were carried out per data point. See the Experimental Section for details.

shown in Figure 1 b, nontoxic concentrations of **1** (n = 18) as low as 4 μmol L^{-1} inhibit proliferation. The inhibition curve shows a half-maximal inhibitor concentration (IC_{50}) of 4.8 μmol L^{-1}, which is well below the LD_{50} value. In view of the detergentlike structure of **1**, cytotoxicity may be attributed to cell lysis and/or the initiation of programmed cell death (apoptosis),[15] which is characterized biochemically by the appearance of ordered arrangements of internucleosomal DNA fragments.[16] To further characterize the cell death caused by **1**, we determined the proportion of apoptotic HaCaT cells by measuring the number of cytosolic nucleosomes after treatment of the cells for 20 h with the synthetic ether lipid. At low but antiproliferative concentrations of **1** (n = 18; 1.5, 4.5 and 7.4 μmol L^{-1}), the number of apoptotic cells showed a concentration-dependent increase; the greatest (tenfold) increase was observed for 7.4 μmol L^{-1} (Figure 2). At concentrations of 14.9 and 29.8 μmol L^{-1} there was a marked decrease in the number of apoptotic cells, as

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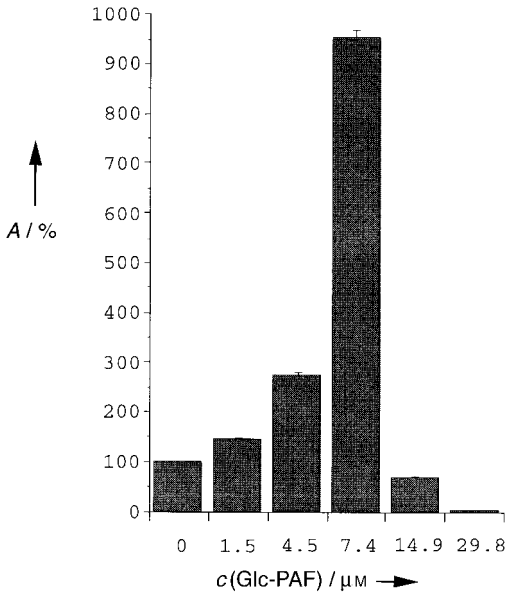

Figure 2. Influence of **1** ($n = 18$) on the apoptosis of HaCaT cells, which is expressed as the percentual enrichment A of apoptotic cells in comparison to the control experiment; c = concentration. Four experiments were carried out per data point. See the Experimental Section for details.

determined by the presence of cytosolic nucleosomes. This indicates that the cells were damaged by lysis at these higher concentrations. According to these data, **1** is a proliferation inhibitor which initiates programmed cell death in keratinocytes at concentrations below 7.5 μmol L^{-1}. Compared with the above-mentioned Glc-PC ($LD_{50} = 17$ μmol L^{-1} and $IC_{50} = 9$ μmol L^{-1} under the experimental conditions described above), **1** ($n = 18$) is not only biologically more active, but also more toxic. The synthesis of other derivatives with 2-glycosidic substituents and different alkyl side chains will be necessary to determine the structure – activity relationships of this new type of compound.

Experimental Section

Cell culture: The spontaneously immortalized human keratinocyte cell line HaCaT[17] was incubated in serum-free keratinocyte growth medium.[18] Compound **1** was dissolved directly in the keratinocyte growth medium.

Cytotoxicity test: Confluent HaCaT cells were incubated for 6 h with different concentrations of **1**. The cytotoxicity of **1** for HaCaT cells was determined in comparison with the control experiments (which did not contain **1**) by measuring the activity of alkaline phosphatase as described.[19]

Measurement of proliferation: HaCaT cells were sown at a cell density of about 20000 cells per cm^2. After adhesion the cells were incubated for 48 h with different concentrations of **1**. The cell count was determined as described[20] after staining with 0.1 % crystal violet in PBS, and compared with the control.

Measurement of apoptosis: Confluent HaCaT cells adapted to keratinocyte growth medium were treated for 20 h with different concentrations of **1**. The medium was removed, and the adherent cells released from the dish with a solution containing 0.1 % trypsin and 0.02 % ethylenediaminetetraacetic acid (EDTA). The cells were then washed off with serum-containing medium (10 % fetal-calf serum in Rosewell Park Memorial Institute (RPMI) medium) and combined with the previously removed medium. The cells were pelleted by centrifugation for 5 min at 300 g, washed once with serum-containing RPMI medium, and pelleted again. The cell pellet was incubated for 30 min in 1 mL of lysis buffer, and the resulting lysate centrifuged for 10 min at 13000 g to remove nonbound antibodies. Nucleosomes were then determined in the lysate and in the control with a commercially available enzyme-linked immunosorbent assay (ELISA) from Boehringer Mannheim according to the instructions provided by the manufacturer. An aliquot of each sample (20 μL) was pipetted into a well of a streptavidin-coated microtiter plate followed by immune reagent (80 μL; made up of 1 part peroxidase-conjugated anti-DNA antibody, 1 part biotinylated anti-histone antibody, and 18 parts incubation buffer) and incubated for 2 h at room temperature. After threefold washing incubation buffer, and the quantity of bound peroxidase-conjugated anti-DNA antibody was quantified by a peroxidase-catalyzed color reaction with measurement at 405 nm in the ELISA reader. The increase in the number of apoptotic cells was calculated by comparing the extinction of the treated sample with that of the corresponding control. The specificity of the method was tested by applying it to cells treated with suitable apoptosis-inducing substances (cell membrane-penetrating ceramides). In addition, electron microscopy of the treated cells revealed typical apoptotic changes, such as membrane evaginations and chromatin condensations, which were correlated with the results of the ELISA test.

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[13] At this stage the anomers were separated by HPLC ($MeOH/CH_2Cl_2$ 4/1). The final product **1** is the pure α-anomer
[14] Selected physical properties and spectroscopic data of **1** ($n = 18$): colorless, amorphous solid; m.p. 225 °C, $[\alpha]_D^{20} = +50.9$ ($c = 0.7$ in CH_3OH); ^1H NMR (500 MHz, CD_3OD): $\delta = 0.90$ (t, $J = 6.25$ Hz, 3 H), 1.18 – 1.38 (m, 30 H), 1.56 (m_c, 2 H), 3.20 (s, 9 H), 3.31 – 3.49 (m, 4 H), 3.58 – 3.80 (m, 8 H), 3.95 – 4.08 (m, 3 H), 4.30 (m_c, 2 H), 5.06 (d, $J =$ 3.75 Hz, 1 H); MS (FAB^+): m/z (%): 672 (4.0), 510 (5.6), 224 (9.4), 184 (39.4), 166 (26.4), 86 (100), 58 (65.7).
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