Synthesis, Spectroscopy, and Cytotoxicity of Glycosylated Acetogenin Derivatives as Promising Molecules for Cancer Therapy[1]

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Several glycosyl derivatives of squamocin (**1**) have been synthesized by glycosylation under Lewis acid catalysis with two different 1-*O*-acetyl sugars. Separation of these compounds has been achieved by HPLC and centrifugal partition chromatography (CPC). A detailed NMR, ESIMS, and LSIMS study allowed complete structural elucidations. The cytotoxic activity of the glycosyl derivatives was investigated and compared with that of squamocin and dihydrosquamocin against human epidermoid carcinoma cells (KB), African green monkey (*Cercopithecus aethiops*) kidney epithelial cells (VERO), and mouse lymphocytic leukemia cells (L1210). The antiproliferative effects of some derivatives were studied on cell cycles in mouse lymphocytic leukemia cells (L1210).

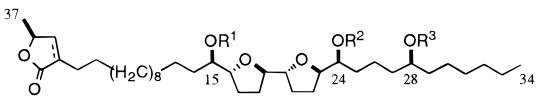
Introduction

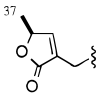
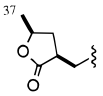
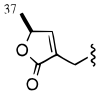
Several species of the Annonaceae family yield a group of secondary metabolites known as annonaceous acetogenins. These polyketides have shown promising anticancer activity as well as antiparasitic and pesticidal properties.[2,3] To investigate the influence of aqueous solubility of acetogenins on their cytotoxicity, we prepared a series of glycosylated derivatives (Table 1) of squamocin,[4] one of the most active and most widespread acetogenins. The biological efficacy of most antitumor drugs is limited to various degrees by side effects resulting from lack of selectivity as well as poor aqueous solubility. Glycosylation often improves solubility in the blood of various bioactive molecules without affecting their activity.[5] The improvement of solubility is a notable advantage in the case of the nonionizable and nonwater-soluble cytotoxic compounds and could allow the administration of aqueous solutions of these drugs. The corresponding glycosides may also act as prodrugs by increasing the bioavailability of various compounds,[6] and thus, acetogenins could be selectively released in the target cells by acidic hydrolyses or enzymatic cleavages.

Results and Discussion

Chemistry. Squamocin **(1)** is a rather complex target for glycosylation due to the presence of three OH groups at C-15, C-24, and C-28. Our aim was to prepare well-defined compounds that could be used for biological screenings. Glycosylation of squamocin was, thus, performed by two approaches (Scheme 1), in which we used 1-*O*-acetyl sugars under Lewis acid catalysis.[7] The first route uses the 1-acetyl-2,3,4,6-tetrabenzyl-α-D-glucopyranose as a glycosyl donor.

Table 1 [a]



	R[1]	R[2]	R[3]	lactone terminus
Squamocin (1)	H	H	H	
Gly-1	H	α-glucose-Bn	H	
Gly-2	H	β-glucose-Bn	H	
Gly-3	α-glucose-Bn	H	H	
Dihydro-squamocin	H	H	H	
Gly-1-D	H	α-glucose	H	
Gly-2-D	H	β-glucose	H	
Gly-3-D	α-glucose	H	H	
Gly-4	β-glucose-Ac	β-glucose-Ac	H	
Gly-5	Ac	β-glucose-Ac	H	
Gly-6	Ac	Ac	β-glucose-Ac	
Gly-7	Ac	β-glucose-Ac	Ac	
Gly-4-D	β-glucose	β-glucose	H	
Gly-5-D	Ac	β-glucose	H	
Gly-6-D	Ac	Ac	β-glucose	

[a] glucose-Bn, 2,3,4,6-tetrabenzyl-α/β-D-glucopyranose; glucose-Ac, 2,3,4,6-tetraacetyl-β-D-glucopyranose.

Considering the hindrance of the benzyl groups, we were expecting both regioselectivity of the glycosylation and formation of monosubstituted derivatives. Moreover, the glycosyl derivatives of dihydrosquamocin could be obtained by a simple catalytic hydrogenation of the protected compounds. The second approach makes use of pentaacetyl-α-D-glucopyranose as glycosyl donor; this protected sugar should induce good stereoselectivity,

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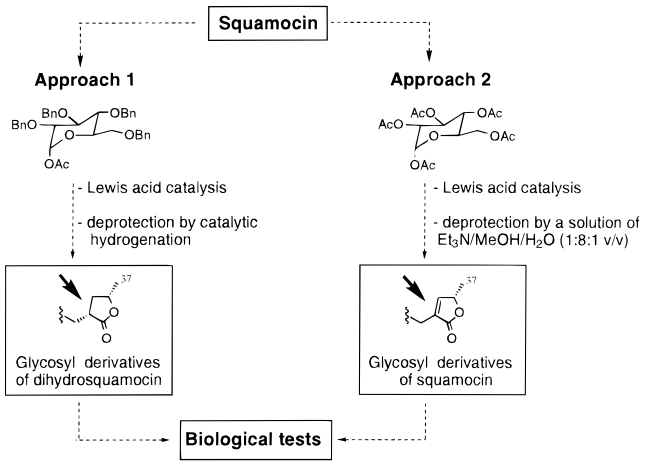
Scheme 1



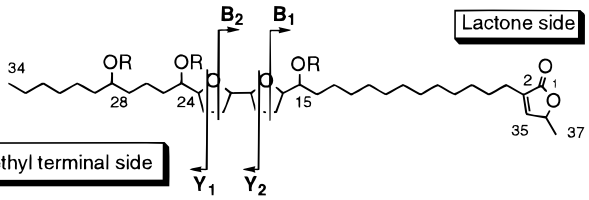
affording the β-anomers, exclusively.[7] Deprotection of the acetyl groups would lead to glycosylated derivatives of squamocin without affecting the lactone ring.

The first glycosylation method using 1-acetyl-2,3,4,6-tetrabenzyl-α-D-glucopyranose as glycosyl donor under Lewis acid catalysis was tested. Optimum conditions ($BF_3{\cdot}Et_2O$, 20 °C, 24 h, CH_2Cl_2/CH_3CN = 1:1) are given in the Experimental Section. Squamocin glycosyl derivatives were obtained and purified by columm chromatography (dichloromethane/MeOH = 90:10). The acetogenin-containing fractions were checked by TLC and further purified by HPLC. Three squamocin glucosyl derivatives (Gly-**1**, Gly-**2,** and Gly-**3**) were isolated. The benzylated glucoside compounds (Gly-**1**, Gly-**2,** and Gly-**3**) were then debenzylated by catalytic hydrogenation, performed in a methanolic solution with 10% Pd/C, at room temperature under H_2 atmospheric pressure, for 2 h, affording Gly-**1**-D, Gly-**2**-D, and Gly-**3**-D in quantitative yields.[8] It is noteworthy that the terminal γ-methyl-α,β-unsaturated lactone of the three compounds was hydrogenated under those conditions.

The second glycosylation method was performed with the pentaacetyl-α-D-glucopyranose as donor, under $BF_3{\cdot}Et_2O$ catalysis. It is important to note that the stereoselective reaction gave exclusively the β-anomers.[7] Squamocin glucosides were purified by centrifugal partition chromatography (CPC), using a quaternary system of heptane/EtOAc/MeOH/H_2O (2:1:2:1) (v/v/v/v), because of the different partition coefficients observed for the glycosyl derivatives by TLC.[9] Four squamocin glucosides (Gly-**4**, Gly-**5,** Gly-**6,** and Gly-**7)** were, thus, isolated. The protecting acetyl groups were then removed by Et_3N/H_2O/MeOH (1:8:1) (v/v/v) treatment,[10] affording Gly-**4**-D, Gly-**5**-D, Gly-**6**-D, and Gly-**7**-D. Surprisingly some compounds obtained by this approach (Gly-**5**/Gly-**5**-D, Gly-**6**/Gly-**6**-D, and Gly-**7)** showed additional acetyl groups in the aliphatic chain.

Mass Characterization. Mass spectrometry of all glycosylated derivatives was performed using LSIMS (liquid secondary ion mass spectrometry) and ESIMS (electrospray ionization mass spectrometry). The molecular weights of the compounds synthesized by the first approach were established by LSIMS as 1144 Da for all three compounds, corresponding to the addition of only one tetrabenzylated glucose to squamocin. The $[M + Li]^+$ ions were observed at *m/z* 1151 in all cases.

Scheme 2



R = H (squamocin **1**), glucose-Bn, glucose-Ac, glucose or Ac

The position of the sugar moiety in the aliphatic chain of Gly-**1**, Gly-**2,** and Gly-**3** was subsequently determined by tandem mass spectrometry.[11] The high-energy collision-induced dissociation (CID) spectrum of the $[M + Li]^+$ ion displayed the typical fragmentation pattern of lithiated acetogenins (Scheme 2).[12]

Two pairs of fragment ion peaks at *m/z* 329/399 and 743/813 were easily assigned to fragmentations across two adjacent THF rings (ions $B_1{-}B_2$ and $Y_1{-}Y_2$, respectively, according to Laprévote and Das),[12] indicating the position of the sugar moiety on the methyl-terminal side chain for Gly-**1** and Gly-**2.** In all CID-MS/MS spectra we observed a main fragmentation, with two ions at *m/z* 543 and 611 corresponding to a cleavage between the squamocin and the sugar moiety; the ion at *m/z* 543 corresponds to the perbenzylated sugar and the ion at *m/z* 611 to squamocin with an unsaturation on the alkyl chain. The MS/MS of the *m/z* 611 ion was very instructive for both the unambiguous location of the sugar moiety in Gly-**1** and Gly-**2** and the location of the unsaturation in the alkyl chain. The high-energy CID spectrum of the *m/z* 611 ion showed two series of fragment ion peaks which were, indeed, attributed to the charge-remote fragmentations of the alkyl chain. Among them, the diagnostic fragment ions at *m/z* 203 (Y_1) and 273 (Y_2) were indicative of the presence of an unsaturation between the THF ring and the terminal methyl goup of the side chain, and the fragment ions at *m/z* 467 and 441 clearly indicated the presence of a double bond at position C-24/C-25 for both compounds. The position of the benzylated glucose was, thus, deduced and attributed to the C-24 position for Gly-**1** and Gly-**2**. The CID spectrum of the $[M + Li]^+$ ion from Gly-**3** displayed the typical fragmentation pattern of lithiated acetogenins.[12] The fragments *m/z* 921/851 and 221/291 assigned to fragmentations across THF rings (ions $B_1{-}B_2$ and $Y_1{-}Y_2$, respectively, according to Laprévote and Das)[12] indicated the position of the sugar moiety on the lactone side chain at C-15.

In the second glycosylation method, the molecular weights were established by ESIMS: Gly-**4** (*m/z* 1305.5 $[M + Na]^+$), Gly-**5** (*m/z* 1017.4 $[M + Na]^+$), Gly-**6** (*m/z* 1059.4 $[M + Na]^+$), and Gly-**7** (*m/z* 1059.4 $[M + Na]^+$). The position of the sugar moiety (Gly-**4**, Gly-**5,** Gly-**6,** and Gly-**7**) was subsequently determined by tandem mass spectrometry of Gly-**4**-D, Gly-**5**-D, and Gly-**6**-D after removal of the glycosyl acetyl groups. The high-energy CID spectrum of the $[M + Li]^+$ ion obtained by LSIMS displayed the typical fragmentation pattern of lithiated acetogenins.[12] The $[M + Li]^+$ ion at *m/z* 953.8 obtained by LSIMS from Gly-**4**-D showed the addition of two sugars to squamocin. The CID spectral data showed two pairs of fragment ion peaks at *m/z* 491/561 and 383/453 which were easily assigned to ions $B_1{-}B_2$

and Y_1-Y_2, respectively, thus indicating the position of one sugar moiety on the lactone side (C-15) and another sugar in the methyl-terminal side chain. The fragments at *m/z* 867 and 675 indicated the position of the second sugar at C-28. The ion at *m/z* 833 $[M + Li]^+$ obtained in LSIMS of Gly-**5**-D showed the addition of one sugar and one acetyl group on squamocin. The high-energy CID spectrum of the $[M + Li]^+$ ion showed two pairs of fragment ion peaks at *m/z* 383/453 and 371/441 (ions B_1-B_2 and Y_1-Y_2, respectively), indicating the position of one sugar in the methyl-terminal side chain and one acetyl group in the lactone side chain (C-15). The fragments at *m/z* 675 and 483 allowed us to locate the sugar at C-24, and fragments at *m/z* 747 and 717 allowed us to position a hydroxyl group at C-28.

The *m/z* 875 $[M + Li]^+$ ion obtained by LSIMS of Gly-**6**-D revealed the addition of one sugar and two acetyl groups to squamocin. The high-energy CID spectrum of the $[M + Li]^+$ ion showed two pairs of fragment ion peaks at *m/z* 425/495 and 371/441, indicating the position of one sugar moiety in the methyl-terminal side chain together with one acetyl group. The fragment ions at *m/z* 789 and 597, separated by 192 Da, confirmed the presence of the sugar at C-28, and the two remaining acetyl groups were thus placed at C-24 and C-15.

The *m/z* 1059.4 $[M + Na]^+$ ion obtained by ESIMS of Gly-**7** indicated the addition of one sugar and two acetyl groups to squamocin. The high-energy CID spectrum of the $[M + Na]^+$ ion showed two pairs of fragment ion peaks at *m/z* 457/387 and 609/679, indicating the position of the sugar unit and one acetyl group in the methyl-terminal side chain, and another acetyl group in the lactone side chain (C-15). Unfortunately, the fragments between the THF rings and the methyl-terminal side chain were of low intensity. It was, thus, impossible to determine unambiguously the position of the substituents on the alkyl chain. However, Gly-**7** is one positional Gly-**6** isomer, and the two compounds have the same substitution (an acetyl group) at C-15. According to the C-24 position of the sugar in Gly-**6**, we, thus, concluded the C-28 position of the glucosyl unit in Gly-**7**.

^{1}H NMR Characterization. ^{1}H NMR spectra recorded at 400 MHz were used for characterization of compounds Gly-**1**/Gly-**7** and Gly-**1**-D/Gly-**6**-D. The detailed resonance assignments are based on integration and selective homonuclear decoupling, as well as 2D homonuclear (COSY DQF) and heteronuclear (HMBC, HMQC) experiments. The Gly-**1**, Gly-**2**, and Gly-**3** ^{1}H NMR spectra showed, in addition to the characteristic pattern of the acetogenin, the presence of the aromatic and methylenic protons due to the benzyl groups. The anomeric carbon configuration was evidenced from the vicinal coupling constants between the C-1′ and C-2′ protons of the sugar. It was deduced as α for Gly-**1** and Gly-**3** ($J_{1'-2'} = 3.7$ Hz) and β for Gly-**2** ($J_{1'-2'} = 7.9$ Hz).[10] In the second approach using pentaacetylglucopyranose, the expected β-configuration ($J_{1'-2'} = 7.9/8.0$ Hz) was observed for all compounds (Gly-**4**, Gly-**5**, Gly-**6**, and Gly-**7**).

Biological Assays. The in vitro cytotoxic activity was investigated (Table 2) against human epidermoid carcinoma cells (KB),[13] African green monkey (*Cercopithecus aethiops*) kidney epithelial cells (VERO),[13] and mouse lymphocytic leukemia cells (L1210).[14] The glucosyl derivatives displayed a significantly higher activity on cancer cell lines (KB, L1210) compared with normal cells (VERO). The remarkable difference in the cytotoxicity toward the KB cells observed for the two compounds Gly-**1**-D and Gly-**1** (Gly-**1**-D being 200 times more active than Gly-**1**) confirms that the presence of the free hydroxyl groups is required for activity.[15] The comparison of the cytotoxic activity against L1210 between Gly-**1**-D (EC_{50} 0.01 μM) and Gly-**2**-D (EC_{50} 0.08 μM) shows that the nature of the sugar bond (α- or β-anomers) does not contribute to the cytotoxic activity. However it is very interesting to note the large decrease in bioactivity for Gly-**4**-D (KB: EC_{50} 1.05 μM; L1210: EC_{50} 10 μM), the only water-soluble derivative (14.8 g/L) substituted by two glycosyl groups.

Table 2. Cytotoxic Activity of Glycosyl Derivatives of Squamocin and Dihydrosquamocin against KB, VERO, and L1210 Cells

compound	EC_{50} (μM)		
	KB[a]	VERO[b]	L1210[c]
Gly-**1**	0.43	0.87	
Gly-**2**			0.05
Gly-**1**-D	0.0019	0.6	0.01
Gly-**2**-D			0.08
dihydrosquamocin	0.00024	0.016	<0.00025
Gly-**4**-D	1.05	1.05	10
Gly-**5**-D	0.36	1.21	50
Gly-**6**	0.00096	0.02	0.3
Gly-**6**-D	0.011	0.34	0.017
squamocin (**1**)	0.000016	0.016	<0.0004
vinblastine*	0.0012	<3.7	

*Reference compound. [a] Human epidermoid carcinoma cells. [b] African green monkey (*C. aethiops*) kidney epithelial cells. [c] Mouse lymphocytic leukemia cells.

This weak activity can be related to an excessive degree of hydroxylation, as previously reported for hexahydroxylated and heptahydroxylated acetogenins.[16,17] On the other hand, the biological activity of Gly-**6** was very surprising, since this compound without any free hydroxyl group was the most cytotoxic glycosyl derivative (Table 2). After deprotection (Gly-**6**-D) the cytotoxic activity was decreased against KB cell lines (EC_{50} 0.011 μM) and increased against L1210 cell lines (EC_{50} 0.017 μM). Perhaps the unexpected cytotoxic activity of the glucosyl derivative Gly-**6** could be rationalized by involving both a different bioavailability and a slow hydrolysis of the acetyl groups in the biological medium containing esterases.

Recently, Raynaud et al. have demonstrated that squamocin was more a cytostatic than a cytotoxic agent toward both sensitive and multidrug-resistant human breast adenocarcinoma cell lines.[18a] The major interest of this antimetabolic agent consists of its prolonged biological effect (from 2 h to 10 days) on cancer cells. Moreover the same study showed that squamocin seems not to be recognized by the plasma membrane glycoproteins (P-glycoprotein); this observation is relatively uncommon for natural products. In this present study, cell cycle inhibition analysis showed that Gly-**2**-D and Gly-**3** inhibit proliferation of the cell line in the G_1 phase (Table 3). These results clearly showed furthermore that glycosyl squamocin derivatives are more specific than the parent natural product.

In conclusion, we have prepared several glycosyl derivatives of squamocin, one of the most active aceto-

Table 3. Cell Cycle Inhibition Activity of Squamocin (**1**), Gly-**2**-D, and Gly-**3** in Mouse Lymphocytic Leukemia Cells (L1210)

compound	cell cycle alterations
squamocin (**1**)	NS[a]
Gly-**2**-D	G_1+[b]
Gly-**3**	G_1++[c]

[a] Not specific (<40%). [b] 54% at 10 μM. [c] 65% at 100 μM.

genins of Annonaceae. It is worth noting that the parent natural product also displays cytotoxicity against non-tumor VERO cells with EC_{50} 16 nM. On their own, glycosyl derivatives showed low activity against VERO cells (EC_{50} from 0.02 to 1.21 μM) while still active against KB (Gly-**6**: EC_{50} 0. 96 nM) or L1210 (Gly-**6**-D: EC_{50} 17 nM). The water soluble derivative Gly-**4**-D, having two sugars, was the less active compound, probably not lipophilic enough to cross the cell membrane, whereas Gly-**6** possessing all protected hydroxyl groups was the most active. Finally, two glycosyl derivatives have shown significant inhibition of the proliferation of L1210 in the G_1 phase, whereas squamocin was not specific. More studies are nevertheless required in order to demonstrate the potency of the glycosylated acetogenins on experimental tumors in vivo, as well as their mechanism(s) of action in vitro.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Philips PU 8700 series UV/Vis spectrophotometer. ^{1}H and ^{13}C NMR spectra were registered at 200 and 50 MHz, respectively, on a Bruker AC-200 P spectrometer and the ^{1}H−^{1}H (COSY-DQF, HOHAHA) and ^{1}H−^{13}C (HMQC, HMBC) correlation spectra at 400 MHz, on a Bruker ARX-400 spectrometer. Chemical shifts are reported as δ values (in parts per million), relative to the residual nondeuterated solvent signals as internal standard. The splitting pattern abbreviations are as follows: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet. Optical rotations were determined using a Schmidt-Haensch Polartronic I polarimeter. HPLC was carried out with a Millipore-Waters (Milford, MA) system equipped with a Waters 484 spectrophotometer. MS and MS/MS spectra were obtained from a Zabspec-T five-sector tandem spectrometer (Micromass, VG Organic, Manchester, U.K.) using the experimental conditions described earlier.[19] CPC were performed using a centrifugal partition chromatograph model LLB (SANKI Engineering Ltd., Kyoto, Japan), equipped with a Waters 600 pump, a Rheodyne 7725i injector (with a 15-mL sample loop), and a model Retriever 500 ISCO collector.

Squamocin. Squamocin **(1)** was isolated from the seeds of *Annona spinescens* Mart.,[20] the bark of *Annona salzmanii* D.C.,[21] and the seeds of *Annona atemoya*:[22] transparent solid; $[\alpha]^{20}_D$ +10° (*c* 1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 209 (4.7) nm; IR ν_{max} (film) 3678, 2994, 1750 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, J = 6.5 Hz), 1.53 (m, CH_2-4), 1.20−1.30 (m, CH_2-5−13), 1.39 (m, CH_2-14), 3.36 (m, CH-15), 3.90 (m, CH-16), 1.50−1.93 (m, $(CH_2)_2$-17,18), 3.90 (m, CH-19,20), 1.50−1.93 (m, $(CH_2)_2$-21,22), 3.90 (m, CH-23), 3.90 (m, CH-24), 1.40 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.44 (m, CH_2-27), 3.59 (m, CH-28), 1.44 (m, CH_2-29), 1.20−1.30 (m, CH_2-30−33), 0.89 (t, CH_3-34, J = 6.5 Hz), 6.97 (d, CH-35, J = 1.5 Hz), 4.99 (qd, CH-36, J = 6.7, 1.7 Hz), 1.42 (d, CH_3-37, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.3 (C-1), 134.0 (C-2), 25.0 (C-3), 74.1 (C-15), 83.1 (C-16), 82.7 (C-19), 82.1 (C-20), 82.5 (C-23), 71.3 (C-24), 32.9 (C-25), 37.2 (C-27/C-29), 71.7 (C-28), 31.5 (C-32), 22.6 (C-33), 14.0 (C-34), 148.9 (C-35), 77.2 (C-36), 19.0 (C-37); CIMS (NH_4^+) m/z 623 $[M + H]^+$; LSIMS (matrix: *m*-nitrobenzyl alcohol + LiCl) m/z 629 $[M + Li]^+$; LSIMS/MS of the $[M + Li]^+$ ion (m/z 629) (series A from the terminal methyl side) 613, 599, 585, 571, 557, 543, 513, 499, 485, 471, 441, 399 (B_2), 329 (B_1), (series B from the lactone side) 531, 517, 503, 489, 475, 461, 447, 433, 419, 405, 391, 377, 363, 333, 291 (Y_2), 221 (Y_1).

Glycosylation of Squamocin. Approach 1: Squamocin **(1)** was dried prior to glycosylation by azeotropic distillation, at atmospheric pressure, with toluene to remove crystal-bound water; compound **1** (0.04 g) was dissolved in a total of 20 mL of a 1:1 anhydrous nitromethane/toluene mixture, and the solvents were distilled to remove traces of water. Dry squamocin **(1)** (0.04 g, 0.06 mmol, 1 equiv) and 1-acetyl-2,3,4,6-tetrabenzyl-α-D-glucopyranose (0.49 g, 0.7 mmol, 12 equiv) were dissolved in 2 mL of CH_2Cl_2:CH_3CN (1:1, v/v) at room temperature under N_2, and BF_3·Et_2O (0.38 mmol, 6 equiv) was quickly added to the vigorously stirred solution. When the reaction was complete (24 h) the mixture was poured into ice-cold saturated aqueous $NaHCO_3$ solution and twice extracted with 10 mL of dichloromethane. Organic layers were dried over Na_2SO_4, filtered and evaporated, and the crude mixture (400 mg) was purified by columm chromatography (silica gel 60M, 230−400 mesh, eluting with CH_2Cl_2:EtOAc = 80:20 v/v). A mixture of three squamocin glycosides (Gly-**1**, Gly-**2,** and Gly-**3**) was obtained. The squamocin glycosides were further purified by HPLC, using a μBondapak C18 prepacked column (10 μm, 25 × 100 mm) and UV detection at 214 nm, elution with MeOH−H_2O (95:5), flow rate 10 mL/min, to afford Gly-**1** (15.4 mg, 37%, t_R = 21.5 min), Gly-**2** (14.6 mg, 34%, t_R = 23.8 min), Gly-**3** (12.3 mg, 28%, t_R = 24.8 min), and squamocin **(1)** (7 mg, 18%, t_R = 15 min).

24-Tetrabenzyl-α-D-glucosylsquamocin (Gly-1): $[\alpha]^{20}_D$ +23° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, J = 6.5 Hz), 1.53 (m, CH_2-4), 1.20−1.30 (m, CH_2-5−13), 1.38 (m, CH_2-14), 3.36 (m, CH-15), 3.90 (m, CH-16), 1.50−1.90 (m, $(CH_2)_2$-17,18), 3.90 (m, CH-19,20), 1.50−1.90 (m, $(CH_2)_2$-21,22), 3.85 (m, CH-23), 3.82 (m, CH-24), 1.40 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.44 (m, CH_2-27), 3.55 (m, CH-28), 1.44 (m, CH_2-29), 1.20−1.30 (m, CH_2-30−33), 0.89 (t, CH_3-34, J = 6.5 Hz), 6.97 (d, CH-35, J = 1.5 Hz), 4.99 (qd, CH-36, J = 6.7, 1.7 Hz), 1.42 (d, CH_3-37, J = 6.6 Hz), 4.65 (d, CH-1′, J = 3.7 Hz), 4.48 (dd, CH-2′, J = 3.9, 10 Hz), 4.80 (m, CH-3′), 4.93 (m, CH-4′), 4.72 (m, CH-5′), 3.40−3.67 (m, CH_2-6′), 4.60−4.90 (m, -*CH_2*-Ph, 2′, 3′, 4′, 6′), 7.20−7.40 (m, -CH_2-*Ph*, 2′, 3′, 4′, 6′); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.2 (C-1), 134.0 (C-2), 25.1 (C-3), 74.1 (C-15), 83.0 (C-16), 82.7 (C-19), 82.1 (C-20), 82.5 (C-23), 80.1 (C-24), 37.0 (C-27/C-29), 71.7 (C-28), 14.2 (C-34), 148.9 (C-35), 77.1 (C-36), 19.0 (C-37), 97.9 (C-1′), 70.7 (C-2′), 72.1 (C-3′), 71.7 (C-4′), 72.3 (C-5′), 62.5 (C-6′), 127.2−130.1 (-CH_2-*Ph*); LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) m/z 1151 $[M + Li]^+$; LSIMS/MS of the $[M + Li]^+$ ion (m/z 1151) (series A from the terminal methyl side) 1093, 1079, 1065, 963, 399 (B_2), 329 (B_1), (series B from the lactone side) 1039, 1025, 1011, 997, 983, 969, 955, 941, 927, 913, 899, 885, 855, 813 (Y_2), 743 (Y_1); LSIMS/MS of the $[M - sugar + Li]^+$ ion (m/z 611) (series A from the terminal methyl side) 595, 581, 567, 553, 539, 525, 495, 481, 467, 441, 399 (B_2), 329 (B_1), (series B from the lactone side) 513, 499, 485, 471, 457, 443, 429, 415, 401, 387, 373, 359, 345, 315, 273 (Y_2), 203 (Y_1).

24-Tetrabenzyl-*β*-D-glucosylsquamocin (Gly-2): $[\alpha]^{20}_D$ +18° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, J = 6.5 Hz), 1.53 (m, CH_2-4), 1.20−1.30 (m, CH_2-5−13), 1.38 (m, CH_2-14), 3.36 (m, CH-15), 3.90 (m, CH-16), 1.50−1.90 (m, $(CH_2)_2$-17,18), 3.90 (m, CH-19,20), 1.50−1.90 (m, $(CH_2)_2$-21,22), 3.90 (m, CH-23), 3.82 (m, CH-24), 1.40 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.44 (m, CH_2-27), 3.55 (m, CH-28), 1.44 (m, CH_2-29), 1.20−1.30 (m, CH_2-30−33), 0.89 (t, CH_3-34, J = 6.5 Hz), 6.97 (d, CH-35, J = 1.5 Hz), 4.99 (qd, CH-36, J = 6.7; 1.7 Hz), 1.42 (d, CH_3-37, J = 6.6 Hz), 4.30 (d, CH-1′, J = 7.9 Hz), 4.21 (dd, CH-2′, J = 7.9, 10 Hz), 4.14 (m, CH-3′), 4.03 (m, CH-4′), 3.46 (m, CH-5′), 3.30−3.53 (m, CH_2-6′), 4.30−5.00 (m, -*CH_2*-Ph, 2′, 3′, 4′, 6′), 7.20−7.40 (m, -CH_2-*Ph*, 2′, 3′, 4′, 6′); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.0 (C-1), 134.0 (C-2), 25.0 (C-3), 74.3 (C-15), 83.2 (C-16), 82.7 (C-19), 82.1 (C-20), 82.4 (C-23), 80.3 (C-24), 37.1 (C-27/C-29), 71.7 (C-28), 14.0 (C-34), 148.6 (C-35), 77.0 (C-36), 19.3 (C-37), 101.5 (C-1′), 71.3 (C-2′), 72.5 (C-3′), 68.4 (C-4′), 71.7 (C-5′), 61.9 (C-6′), 127.4−130.0 (-CH_2-*Ph*);

LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 1151 [M + Li]$^+$; LSIMS/MS of the [M + Li]$^+$ ion (*m/z* 1151) (series A from the terminal methyl side) 1093, 1079, 1065, 963, 399 (B_2), 329 (B_1), (series B from the lactone side) 1039, 1025, 1011, 997, 983, 969, 955, 941, 927, 913, 899, 885, 855, 813 (Y_2), 743 (Y_1); LSIMS/MS of the [M − sugar + Li]$^+$ ion (*m/z* 611) (series A from the terminal methyl side) 595, 581, 567, 553, 539, 525, 495, 481, 467, 441, 399 (B_2), 329 (B_1), (series B from the lactone side) 513, 499, 485, 471, 457, 443, 429, 415, 401, 387, 373, 359, 345, 315, 273 (Y_2), 203 (Y_1).

15-Tetrabenzyl-α-D-glucosylsquamocin (Gly-3): $[\alpha]^{20}_D$ +22.5° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, *J* = 6.5 Hz), 1.53 (m, CH_2-4), 1.20−1.30 (m, CH_2-5−13), 1.38 (m, CH_2-14), 3.45 (m, CH-15), 3.79 (m, CH-16), 1.50−1.90 (m, $(CH_2)_2$-17,18), 3.90 (m, CH-19,20), 1.50−1.90 (m, $(CH_2)_2$-21,22), 3.85 (m, CH-23), 3.82 (m, CH-24), 1.40 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.44 (m, CH_2-27), 3.55 (m, CH-28), 1.44 (m, CH_2-29), 1.20−1.30 (m, CH_2-30−33), 0.89 (t, CH_3-34, *J* = 6.5 Hz), 6.97 (d, CH-35, *J* = 1.5 Hz), 4.99 (qd, CH-36, *J* = 6.7, 1.7 Hz), 1.42 (d, CH_3-37, *J* = 6.6 Hz), 4.65 (d, CH-1′, *J* = 3.7 Hz), 4.49 (dd, CH-2′, *J* = 3.9, 10 Hz), 4.80 (m, CH-3′), 4.95 (m, CH-4′), 4.70 (m, CH-5′), 3.40−3.65 (m, CH_2-6′), 4.60−4.90 (m, -*CH*$_2$-Ph, 2′, 3′, 4′, 6′), 7.20−7.40 (m, -CH_2-*Ph*, 2′, 3′, 4′, 6′); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.0 (C-1), 134.0 (C-2), 25.2 (C-3), 78.6 (C-15), 83.0 (C-16), 82.6 (C-19), 82.1 (C-20), 82.5 (C-23), 71.4 (C-24), 37.1 (C-27/C-29), 82.8 (C-28), 14.0 (C-34), 148.8 (C-35), 77.0 (C-36), 19.0 (C-37), 97.7 (C-1′), 70.5 (C-2′), 72.0 (C-3′), 71.5 (C-4′), 72.2 (C-5′), 62.5 (C-6′), 127.0−130.1 (-CH_2-*Ph*); LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 1151 [M + Li]$^+$; LSIMS/MS of the [M + Li]$^+$ ion (*m/z* 1151) (series A from the terminal methyl side) 1093, 1079, 1065, 963, 921 (B_2), 851 (B_1), (series B from the lactone side) 1039, 1025, 1011, 997, 983, 969, 955, 941, 927, 913, 889, 885, 335, 291 (Y_2), 221 (Y_1).

Deprotection of the Benzylated Glycosyl Groups. Approach 1: The benzylated glucoside compounds Gly-**1**, Gly-**2,** and Gly-**3** were deprotected by catalytic hydrogenation performed in a methanolic solution with 10% Pd/C at room temperature under an atmospheric pressure of H_2 for 2 h. The reaction mixture was filtered using a Sep-Pack C_{18} cartridge affording: Gly-**1**-D (9.5 mg, 87%) LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 789 [M + Li]$^+$, Gly-**2**-D (8.9 mg, 86%) LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 789 [M + Li]$^+$, and Gly-**3**-D (7.1 mg, 84%) LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 789 [M + Li]$^+$.

Glycosylation of Squamocin. Approach 2: Squamocin **(1)** was dried prior to glycosylation by azeotropic distillation at atmospheric pressure with toluene to remove crystal-bound water (as described above). Dry squamocin **(1)** (0.1 g, 0.16 mmol, 1 equiv) and pentaacetyl-α-D-glucopyranose (0.25 g, 0.64 mmol, 4 equiv) were dissolved in CH_2Cl_2 (2 mL) at room temperature under N_2 and $BF_3{\cdot}Et_2O$ (1.4 mmol, 9 equiv) was quickly added to the vigorously stirred solution. When the reaction was complete (24 h) the mixture was poured into ice-cold saturated aqueous $NaHCO_3$ solution and twice extracted with 10 mL of dichloromethane. Organic layers were dried over Na_2SO_4, filtered and evaporated, and the crude mixture (970 mg) was purified by CPC. Experiment was performed with the quaternary system of heptane/EtOAc/MeOH/H_2O (2:1:2:1) (v/v/v/v). The coil was first entirely filled with the upper phase and rotation was set to the desired speed (1100 rpm). The lower phase was then pumped into the column at a flow rate of 3 mL/min in descending mode (mobile phase = lower phase; stationary phase = upper phase). After the equilibrium between the two phases (mobile phase = 59 mL, dead volume V_0 = 50 mL), the sample solution of the reaction mixture (970 mg) in 10 mL of mobile phase was injected through the injector at 1 mL/min flow rate. The flow rate was then gradually increased to 3 mL/min and the effluent was collected from the outlet of the column. After fraction 200 had been collected, the rotation was stopped and the content of the columm was pushed out by MeOH, fractions were checked by TLC (CH_2Cl_2:EtOAc = 80:20), affording Gly-**4** (13.9 mg, 10%), Gly-**5** (18 mg, 17%), Gly-**6** (17.1 mg, 16%), Gly-**7** (8.8 mg, 7.9%), and squamocin **(1)** (20.7 mg, 20%).

15,24-Ditetraacetyl-β-D-glucosylsquamocin (Gly-4): $[\alpha]^{20}_D$ +26° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, *J* = 6.5 Hz), 1.20−1.30 (m, CH_2-4−14), 3.55 (m, CH-15), 3.85 (m, CH-16), 1.55−1.95 (m, $(CH_2)_2$-17,18), 3.90−4.00 (m, CH-19,20), 1.55−1.95 (m, $(CH_2)_2$-21,22), 3.89 (m, CH-23), 3.85 (m, CH-24), 1.20−1.40 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.35−1.45 (m, CH_2-27), 3.70 (m, CH-28), 1.44 (m, CH_2-29), 1.25−1.45 (m, CH_2-30−33), 0.89 (t, CH_3-34, *J* = 6.5 Hz), 6.97 (d, CH-35, *J* = 1.5 Hz), 4.99 (qd, CH-36, *J* = 6.7, 1.7 Hz), 1.42 (d, CH_3-37, *J* = 6.6 Hz), 4.90 (m, CH-1′)*, 4.86−5.20 (m, CH-2′)*, 4.86−5.20 (m, CH-3′)*, 4.86−5.20 (m, CH-4′)*, 4.10−4.20 (m, CH-5′)*, 4.10−4.20 (m, CH_2-6′)*, 2.03, 2.04, 2.05, 2.09 (4s, Ac-2′, 3′,-4′,6′)* (*the same values were obtained for the two sugar moieties); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.0 (C-1), 134.0 (C-2), 79.5 or 79.9 (C-15), 82.9 (C-16), 82.6 (C-19), 82.1 (C-20), 82.4 (C-23), 71.4 (C-24), 37.1 (C-27/C-29), 79.5 or 79.9 (C-28), 14.0 (C-34), 148.8 (C-35), 77.2 (C-36), 19.2 (C-37), 101.5 (C-1′/C-1″), 71.5 (C-2′/C-2"), 72.2 (C-3′/C-3"), 68.4 (C-4′/C-4"), 71.0 (C-5′/C-5"), 62.5 (C-6′/C-6"); ESIMS *m/z* 1305.5 [M + Na]$^+$.

15-Acetyl-24-tetraacetyl-β-D-glucosylsquamocin (Gly-5): $[\alpha]^{20}_D$ +17° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, *J* = 6.5 Hz), 1.20−1.30 (m, CH_2-4−14), 4.86 (m, CH-15), 3.93 (m, CH-16), 1.55−1.90 (m, $(CH_2)_2$-17,18), 3.90−4.00 (m, CH-19,20), 1.55−1.90 (m, $(CH_2)_2$-21,22), 3.88 (m, CH-23), 3.85 (m, CH-24), 1.20−1.30 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.35−1.45 (m, CH_2-27), 3.65 (m, CH-28), 1.35−1.45 (m, CH_2-29), 1.20−1.30 (m, CH_2-30−33), 0.89 (t, CH_3-34, *J* = 6.5 Hz), 6.97 (d, CH-35, *J* = 1.5 Hz), 4.99 (qd, CH-36, *J* = 6.7, 1.7 Hz), 1.42 (d, CH_3-37, *J* = 6.6 Hz), 2.04 (s, Ac-15), 4.85 (d, CH-1′, *J* = 8.0 Hz), 4.94 (dd, CH-2′, *J* = 9.3; 10.1 Hz), 5.19 (m, CH-3′), 5.05 (m, CH-4′), 4.12 (m, CH-5′), 4.20 (m, CH_2-6′), 2.01, 2.06, 2.07, 2.08 (4s, Ac-2′,3′,4′,6′); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.2 (C-1), 134.0 (C-2), 71.7 (C-15), 83.0 (C-16), 82.5 (C-19), 81.9 (C-20), 82.5 (C-23), 79.6 (C-24), 37.0 (C-27/C-29), 71.6 (C-28), 14.0 (C-34), 148.9 (C-35), 77.0 (C-36), 19.2 (C-37), 170.6/21,3 (OAc-15), 101.3 (C-1′), 71.3 (C-2′), 72.5 (C-3′), 68.0 (C-4′), 71.5 (C-5′), 62.1 (C-6′); chemical shifts observed between 169.5 and 171.0 ppm and between 21 and 21.5 ppm were attributed to the carbon atoms of four acetyl groups; ESIMS *m/z* 1017.4 [M + Na]$^+$.

15,24-Diacetyl-28-tetraacetyl-β-D-glucosylsquamocin (Gly-6): $[\alpha]^{20}_D$ +18° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, *J* = 6.5 Hz), 1.53 (m, CH_2-4), 1.20−1.30 (m, CH_2-5−14), 4.85 (m, CH-15), 3.94 (m, CH-16), 1.50−2.00 (m, $(CH_2)_2$-17,18), 3.87−4.00 (m, CH-19,20), 1.50−2.00 (m, $(CH_2)_2$-21,22), 3.95 (m, CH-23), 4.93 (m, CH-24), 1.35−1.45 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.44 (m, CH_2-27), 3.55 (m, CH-28), 1.44 (m, CH_2-29), 1.20−1.30 (m, CH_2-30−33), 0.89 (t, CH_3-34, *J* = 6.5 Hz), 6.97 (d, CH-35, *J* = 1.5 Hz), 4.99 (qd, CH-36, *J* = 6.7, 1.7 Hz), 1.42 (d, CH_3-37, *J* = 6.6 Hz), 2.04 (s, Ac-15), 2.05 (s, Ac-24), 4.85 (d, CH-1′, *J* = 8.0 Hz), 4.96 (dd, CH-2′, *J* = 8.0, 9.5 Hz), 5.19 (m, CH-3′), 5.05 (m, CH-4′), 4.12 (dd, CH-5′, *J* = 2.4, 10 Hz), 4.20 (dd, CH_2-6′, *J* = 2.3, 4.5 Hz), 2.01, 2.02, 2.07, 2.08 (4s, Ac-2′,3′,4′,6′); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.2 (C-1), 134.1 (C-2), 71.6 (C-15), 82.9 (C-16), 82.6 (C-19), 82.1 (C-20), 82.4 (C-23), 71.5 (C-24), 36.9 (C-27/C-29), 79.5 (C-28), 14.0 (C-34), 148.8 (C-35), 77.2 (C-36), 19.2 (C-37), 101.3 (C-1′), 71.3 (C-2′), 72.5 (C-3′), 68.0 (C-4′), 71.5 (C-5′), 62.1 (C-6′); chemical shifts observed between 169.5 and 171.5 ppm and between 21.2 and 21.4 ppm were attributed to the carbon atoms of six acetyl groups; ESIMS *m/z* 1059.4 [M + Na]$^+$.

15,28-Diacetyl-24-tetraacetyl-β-D-glucosylsquamocin (Gly-7): ^{1}H NMR ($CDCl_3$, 400 MHz) 2.26 (t, CH_2-3, *J* = 6.5 Hz), 1.53 (m, CH_2-4), 1.20−1.30 (m, CH_2-5−14), 4.85 (m, CH-15), 3.93 (m, CH-16), 1.50−2.00 (m, $(CH_2)_2$-17,18), 3.87−4.00 (m, CH-19,20), 1.50−2.00 (m, $(CH_2)_2$-21,22), 3.90 (m, CH-23), 3.55 (m, CH-24), 1.35−1.45 (m, CH_2-25), 1.20−1.30 (m, CH_2-26), 1.44 (m, CH_2-27), 4.85 (m, CH-28), 1.44 (m, CH_2-29), 1.20−1.30 (m, CH_2-30−33), 0.85 (t, CH_3-34, *J* = 6.5 Hz), 6.96 (d, CH-35, *J* = 1.5 Hz), 4.98 (qd, CH-36, *J* = 6.7, 1.7 Hz), 1.42 (d, CH_3-37, *J* = 6.6 Hz), 2.04 (s, Ac-15), 2.05 (s, Ac-28), 4.84 (d, CH-1′, *J* = 7.9 Hz), 4.95 (dd, CH-2′, *J* = 8.0, 9.5 Hz), 5.17 (m, CH-3′), 5.01 (m, CH-4′), 4.13 (dd, CH-5′, *J* = 2.5, 10 Hz), 4.18

(dd, CH_2-6′, J = 2.1, 4.5 Hz), 2.02, 2.07, 2.08 (3s, Ac-2′,3′,4′,6′); ^{13}C NMR ($CDCl_3$, 50 MHz) 174.1 (C-1), 134.3 (C-2), 71.4 (C-15), 82.7 (C-16), 82.5 (C-19), 82.1 (C-20), 82.3 (C-23), 79.3 (C-24), 36.9 (C-27/C-29), 71.4 (C-28), 14.2 (C-34), 148.7 (C-35), 77.0 (C-36), 19.0 (C-37), 101.0 (C-1′), 71.4 (C-2′), 72.4 (C-3′), 68.02 (C-4′), 71.2 (C-5′), 62.2 (C-6′); chemical shifts observed between 169.6 and 171.8 ppm and between 21.2 and 21.4 ppm were attributed to the carbon atoms of six acetyl groups; ESIMS *m/z* 1059.4 $[M + Na]^+$; ESIMS/MS of the $[M + Na]^+$ ion (*m/z* 1059.4) (series A from the terminal methyl side) 1043, 1029, 1015, 1001, 987, 973, 457 (B_2), 387 (B_1), (series B from the lactone side) 961, 947, 933, 919, 905, 891, 877, 863, 849, 835, 821, 807, 793, 721, 679 (Y_2), 609 (Y_1).

Deprotection of the Acetyl Glycosyl Groups. Approach 2: The deprotection was performed by treatment with the Et_3N:MeOH:H_2O (1:8:1 v/v/v) mixture at 35 °C for 30 h, and squamocin glycosides were filtrated using Sep-Pack C_{18} cartridges, affording, after evaporation, pure compounds, as judged by TLC.

15,24-Di-*β*-D-glucosylsquamocin (Gly-4-D): 12.5 mg, 91%; LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 953.8 $[M + Li]^+$; LSIMS/MS of the $[M + Li]^+$ ion (*m/z* 953.8) (series A from the terminal methyl side) 937, 923, 909, 895, 881, 867, 675, 661, 647, 633, 603, 561 (B_2), 491 (B_1), 463, (series B from the lactone side) 841, 827, 813, 799, 785, 771, 757, 743, 729, 715, 701, 687, 495, 453 (Y_2), 383 (Y_1).

15-Acetyl-24-*β*-D-glucosylsquamocin (Gly-5-D): 8.6 mg, 95.5%; LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 833 $[M + Li]^+$; LSIMS/MS of the $[M + Li]^+$ ion (*m/z* 833) (series A from the terminal methyl side) 817, 803, 789, 775, 761, 747, 717, 703, 689, 675, 483, 441 (B_2), 371 (B_1), 343, (series B from the lactone side) 721, 707, 693, 679, 665, 651, 637, 623, 609, 595, 581, 567, 495, 453 (Y_2), 383 (Y_1).

15,24-Diacetyl-28-*β*-D-glucosylsquamocin (Gly-6-D): 12 mg, 86%; LSIMS (matrix: *m*-nitrobenzyl alcohol + LiI) *m/z* 875.8 $[M + Li]^+$; LSIMS/MS of the $[M + Li]^+$ ion (*m/z* 875.8) (series A from the terminal methyl side) 859, 845, 831, 817, 803, 789, 597, 583, 569, 555, 483, 441 (B_2), 371 (B_1), 343, (series B from the lactone side) 763, 749, 735, 721, 707, 693, 679, 665, 651, 637, 623, 609, 537, 495 (Y_2), 425 (Y_1), 397.

In Vitro Cytotoxicity of Glycosyl Derivatives. The in vitro tests against human epidermoid carcinoma cells (KB) and African green monkey (*C. aethiops*) kidney epithelial cells (VERO) were performed using the procedure described by Quéro et al.[12] Cytotoxicity is expressed as concentrations of the product that cause 50% growth inhibition (EC_{50}). Assays were performed in 96-well culture plates with 3-fold serial dilutions added to a 24-h-old monolayer of KB or VERO cells. After 72 h of incubation at 37 °C in a humidified 5% CO_2 atmosphere, cell monolayers were fixed in formol, stained with methylene blue, and then washed extensively with tap water. Hydrochloric acid (0.1 M) was added to each well and the absorbance was measured (wavelength 620 nm) with a multichannel spectrophotometer. The mean of the absorbance of cells cultured in medium containing acetogenin as a percentage of that of control cells cultured in acetogenin-free medium gave the cell proliferation rate over a period of 72 h. The concentrations of the compounds inducing 50% inhibition of proliferation (EC_{50}) as compared with the control cells were determined from the dose−response curves. The in vitro tests against mouse lymphocytic leukemia cells (L1210) were performed using the procedure described by C. Paoletti et al.[13] Briefly, L1210 lymphocytic leukemia cells were grown in nutrient RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin (200 IU/mL) and streptomycin (50 μg/mL). The cell cultures were tested and shown to be free of any contamination. Under these conditions, the generation time is about 13 h. Exponentially growing cells (1×10^4/well) were incubed in the growth medium with the test drugs at different concentrations (the drugs were first dissolved in dimethyl sulfoxide (DMSO), then diluted with the cell culture medium), at 37 °C for 72 h. Total cell numbers at the different drug concentrations were determined by a Coulter ZM cell counter. The average number of cells in each triplicate experiment was expressed as a percentage of the average number of triplicate controls. The IC_{50} (the concentration that inhibits 50% of cell growth after a 72-h exposure to the drug) was determined from the percentage of the residual cells versus the drug concentration on a semilogarithmic scale. The IC_{50} values were calculated with Prism 2 software (GraphPad Software Inc.).

Cell Cycle Analysis. L1210 cells were cultivated with squamocin at different concentrations (10, 100, 1000 ng/mL for L1210) for 24 h in 25-mL plastic culture flasks (ATGC, Noisy-le-Grand, France). For each cell line, a control flask (squamocin-free medium) was also used. Bromodeoxyuridine (BrdU, Sigma) at a concentration of 30 mM was added after 15 min. The cells were permeabilized with ethanol, labeled with a rat anti-BrdU antibody (Seralab, Sigma), and diluted 1:25 in buffer and subsequently fluorescein isothiocyanate-conjugated goat antibody diluted 1:50 (Cliniscience, Paris, France) was added. Cells were incubated with propidium iodide−PBS (1:50, v/v, Sigma) and subjected to flow cytometry (Brunker). Data were analyzed automatically by a LYSYS program and results expressed as histograms. The percentage of cells present in areas corresponding to each phase of the cell cycle was calculated.

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References

(1) Acetogenins from Annonaceae 84. For part 83, see: Pichon, M.; Hocquemiller, R.; Figadère, B. Synthesis of C1−C32 Fragment of Aza-solamin, an Unnatural Analogue of the Annonaceous Acetogenin Solamin. *Tetrahedron Lett.* **1999**, *40*, 8567−8570.

(2) Cavé, A.; Cortes, D.; Figadère, B.; Hocquemiller, R.; Laprévote, O.; Laurens, A.; Lebœuf, M. Recent Advances in the Acetogenins of Annonaceae. In *Recent Advances in Phytochemistry: Phytochemical Potential of Tropical Plants*; Downum, K. R., Romeo, J. T., Stafford, A. H., Eds.: Plenum Press: New York, 1993; Vol. 27, pp 167−202.

(3) (a) Cavé, A.; Cortes, D.; Figadère, B.; Laurens, A. Acetogenins from Annonaceae. In *Progress in the Chemistry of Organic Natural Products;* Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, Ch., Eds.; Springer: New York, 1997; Vol. 70, pp 81−288. (b) Alali, F. Q.; Liu, X. X. Annonaceous Acetogenins: Recent Progress. *J. Nat. Prod.* **1999**, *62*, 504−540.

(4) Fujimoto, Y.; Eguchi, T.; Kakinuma, K.; Ikekawa, N.; Sahai, M.; Gupta, Y. K. Squamocin, a New Cytotoxic Bis-Tetrahydrofuran Containing Acetogenin from *Annona squamosa*. *Chem. Pharm. Bull.* **1988**, *36*, 4802−4806.

(5) Sherwood, R. F. Advanced Drug Delivery Reviews: Enzyme Prodrug Therapy. *Adv. Drug Delivery Rev.* **1996**, *22*, 269−288.

(6) Florent, J.-C.; Dong, X.; Gaudel, G.; Mitaku, S.; Monneret, C.; Gesson, J.-P.; Jacquesy, J.-C.; Mondon, M.; Renoux, B.; Andrianomenjanahary, S.; Michel, S.; Koch, M.; Tillequin, F.; Gerken, M.; Czech, J.; Straub, R.; Bosslet, K. Prodrugs of Antracyclines for Use in Antiboby-Directed Enzyme Prodrug Therapy. *J. Med. Chem.* **1998**, *41*, 3572−3581.

(7) Toshima, K.; Tatsuda, K. Recent Progress in *O*-Glycosylation Methods and its Application to Natural Products Synthesis. *Chem. Rev.* **1993**, *93*, 1503−1531.

(8) Cortes, D.; Myint, S. H.; Harmange, J. C.; Sahpaz, S.; Figadère, B. Catalytic Hydrogenation of Annonaceous Acetogenins. *Tetrahedron Lett.* **1992**, *33*, 5225−5226.

(9) Foucault, A. *Centrifugal Partition Chromatography*; Chromatography Sciences Series; Marcel Dekker Inc.: New York, 1995; Vol. 68, Chapter 4, pp 71−97.

(10) Kren, V.; Kubisch, J.; Sedmera, P.; Halaba, P.; Prikrylova, V.; Jegorov, A.; Cvak, L.; Gebhardt, R.; Ulrichova, J.; Simanek, V. Glycosylation of Sylibin. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2467−2474.

(11) Queiroz, E. F.; Roblot, F.; Laprévote, O.; Serani, L.; Cavé, A. Spinencin, a New Bis-Tetrahydrofuran Acetogenin from the Seeds of *Annona spinescens*. *J. Nat. Prod.* **1997**, *60*, 760−765.

(12) Laprévote, O.; Das, B. C. Structural Elucidation of Acetogenins from Annonaceae by Fast-Atom Bombardment Mass Spectrometry. *Tetrahedron* **1994**, *50*, 8479−8490.

(13) Fleury, C.; Cotte-Laffitte, J.; Quéro, A.-M. Évaluation de la Cytotoxicité d'un Antiseptique par une Microméthode Photométrique. *Pathol. Biol*. **1984**, *32*, 628−630.
(14) Paoletti, C.; Cros, S.; Dat-Xuong, N.; Leconte, P.; Moisand, A. Comparative Cytotoxic and Antitumoral Effects of Ellipticine Derivatives on Mouse L-1210 Leukemia. *Chem Biol. Interact.* **1979**, *25*, 45−58.
(15) Duret, P.; Hocquemiller, R.; Gantier, J.-C.; Figadère, B. Semisynthesis and Cytotoxicity of Amino Acetogenins and Derivatives. *Bioorg. Med. Chem*. **1999**, *7*, 1821−1826.
(16) Zeng, L.; Wu, F.-E.; McLaughlin, J. L. Annohexocin, a Novel Mono-THF Acetogenin with Six Hydroxyls, from *Annona muricata* (Annonaceae). *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1865−1868.
(17) Meneses da Silva, E. L.; Roblot, F.; Laprévote, O.; Serani, L.; Cavé, A. Coriaheptocins A and B, the First Heptahydroxylated Acetogenins, Isolated from the Roots of *Annona coriacea*. *J. Nat. Prod*. **1997**, *60*, 162−167.
(18) (a) Raynaud, S.; Némati, F.; Miccoli, L.; Michel, P.; Poupon, M.-F.; Fourneau, C.; Laurens, A.; Hocquemiller, R. Antitumoral Effects of Squamocin on Parental and Multidrug Resistant MCF7 (Human Breast Adenocarcinoma) Cell Lines. *Life Sci.* **1999**, *65*, 525−533. (b) For precedent, see: Oberlies, N. H.; Croy, V. L.; Harrison, M. L.; McLaughlin, J. L. The Annonaceous Acetogenin Bullatacin is Cytotoxic Against Multidrug Resistant Human Mammary Adenocarcinoma Cells. *Cancer Lett.* **1997**, *115*, 73.
(19) Gleye, C.; Laurens, A.; Hocquemiller, R.; Cavé, A.; Laprévote, O.; Serani, L. Isolation of Montecristin, a Key Metabolite in Biogenesis of Acetogenins from *Annona muricata* and its Structure Elucidation by Using Tandem Mass Spectrometry. *J. Org. Chem*. **1997**, *63*, 510−513.
(20) Queiroz, E. F.; Roblot, F.; Figadère, B.; Laurens, A.; Duret, P.; Hocquemiller, R.; Cavé, A.; Serani, L.; Laprévote, O.; Cotte-Laffitte, J.; Quéro, A.-M. Three New Bis-Tetrahydrofuran Acetogenins from the Seeds of *Annona spinescens*. *J. Nat. Prod*. **1998**, *61*, 34−39.
(21) Queiroz, E. F.; Roblot, F.; Cavé, A.; Hocquemiller, R.; Serani, L.; Laprévote, O.; Paulo, M. de Q. A New Bis-Tetrahydrofuran Acetogenin from the Roots of *Annona salzmanii*. *J. Nat. Prod*. **1999**, *62*, 710−713.
(22) Duret, P.; Hocquemiller, R.; Laurens, A.; Cavé, A. Atemoyin, a New Bis-Tetrahydrofuran Acetogenin from the Seeds of *Annona atemoya*. *Nat. Prod. Lett*. **1995**, *5*, 295−302.

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