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Semisynthesis and Cytotoxicity of Amino Acetogenins and Derivatives†

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Abstract—Semisynthetic derivatives were prepared from two natural annonaceous acetogenins, rolliniastatin-1 and squamocin, and their cytotoxicity was evaluated. Amino derivatives show decreased bioactivity. Isorolliniastatin-1 was found to be much less toxic than rolliniastatin-1 after intraperitoneal administration to mice, although the in vitro cytotoxicity of both compounds was comparable

Introduction

Since the first isolation of the acetogenin of Annonaceae uvaricin in 1982,[2] more than 300 new natural acetogenins have been isolated and fully characterized from either bark, leaves, or seeds of 30 different Annonaceae species,[3,4,8] and have been found so far to be exclusive to this family. They all have in common, structural features such as a terminal butyrolactone (most frequently α,β-unsaturated) and a long alkyl chain (30 or 32 carbon atoms) substituted by oxygenated functions (e.g. tetrahydrofuran(s), hydroxyl(s), epoxide(s),...). A tentative classification has been proposed,[10] based on biogenetic hypotheses, defined by the presence of one tetrahydrofuran (THF) ring flanked by two hydroxyls for type A, two contiguous THF rings flanked by two hydroxyls for type B, two THF rings separated by four methylenes for type C, three adjacent THF rings for type D, whereas type E is characterized by the lack of any THF ring. Indeed, recent isolation of the $\Delta^{n,n+4}$ and the $\Delta^{n,n+4,n+8}$ di- and tri-unsaturated compounds[11] (type E acetogenins) by our group and others has confirmed the biogenetic hypothesis proposed by several teams. Recently, tetrahydropyran (THP) acetogenins were isolated by McLaughlin et al.[12,13]

Insecticide, antiparasitic and immunosuppressive activities have been reported for some acetogenins.[3–7] Potent cytotoxicity against several cancer cell lines (e.g. EC_{50} of bullatacin against KB cells: 10^{-12} μg mL^{-1})[14] has been observed for many acetogenins. A few of them have been studied in vivo and some have shown promising antitumour activity.[3–7] The antitumour and cytotoxic activity may be explained by the inhibition of NADH oxidases both at the mitochondrial level[9] (complex I of the respiratory chain) and the NADH oxidase located on the cytoplasmic membranes of cancer cells.[15] Inhibition of both enzymes would result in a decreased biosynthesis of ATP and thus decreased cell proliferation (particularly cancer cells which have a high energy requirement) and may ultimately contribute to programmed death (apoptosis).[16] Interestingly, this growth inhibition was also observed for the cancer cells expressing a multi-drug resistant (MDR) phenotype[3,4,17] (with the same range of EC_{50} as for the corresponding sensitive cells[18]). This mechanism of action has no equivalent, to our knowledge, among the anticancer chemotherapeutic agents currently in use.

Since the complex I in mitochondria may be considered as an iron–protein complex,[19] and because Ca^{2+} ions seem to play a role in the expression of NADH oxidases both on plasma membranes and in mitochondria,[16,20] variations in the ability of natural acetogenins and their analogues to complex different cations (K^+, Ba^{2+}, Ca^{2+}) may explain differences in their biological activity.[20–25] However, other factors (e.g. hydrophobic

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† Part 76 in the Acetogenins of Annonaceae: for part 75, see ref 1.

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interactions in the lipid membranes) cannot be excluded when the structure–activity relationships are analyzed.[26]

Preparation of amino acetogenins and derivatives may increase the solubility, the cations complexation ability and/or the hydrogen bonding capacity in lipid membranes.[26] In an attempt to gain more information about the structure–activity relationships of natural annonaceous acetogenins, semisynthesis and biological evaluation of several derivatives, such as compounds **1**–**9**, have been carried out.

Results and Discussion

Many groups have been carrying out the total synthesis of annonaceous acetogenins[27–30] whereas very few semisynthetic compounds have been prepared from the natural products, which are only available in small quantities. Rolliniastatin-1 (**1**) was isolated in a large amount from the seeds of *Rollinia membranacea* and also from seeds of *Annona atemoya*. It is noteworthy that it is the first time, to our knowledge, that **1** has been found in *A. atemoya* species.[31] Isorolliniastatin-1 (**3**) was obtained after 10 h treatment of the parent compound **1** with diethylamine at room temperature.[32] Derivatives **4**–**9** were prepared through chemical transformations that do not affect the very sensitive γ-methyl-α,β-unsaturated-γ-lactone located at the terminus[33] (see Chart 1).

For instance, 4,15,24-trideoxyrolliniastatin-1 (**4**) was obtained in three steps from the parent compound **1** after preparation of the trimesylrolliniastatin-1 intermediate (by treatment of **1** with mesyl chloride in pyridine), followed by treatment with sodium iodide in THF and then reduction of the iodo derivative so formed with tributyltin hydride in the presence of AIBN (Scheme 1). Squamocin **2** was also isolated from the seeds of *A. atemoya*.[34] 15,24,28-Tritosylsquamocin (**5**) and 15,24,28-trimesylsquamocin (**6**) were prepared by treatment of squamocin (**2**) with the appropriate sulfonyl chloride. 15,24,28-Trideoxy-15,24,29-triazidosquamocin (**7**) was then obtained by mixing 15,24,28-trimesylsquamocin (**6**) and sodium azide in DMSO for 15 h at room temperature. It is noteworthy that the absolute configurations at C-15 and C-24 were inverted because of the SN_2 type character of the mesylate displacement reaction by sodium azide. On the other hand, 15,24,28-trideoxy-15,24,29-triaminosquamocin (**8**) was prepared by treatment of 15,24,28-trideoxy-15,24,29-triazidosquamocin (**7**) with triphenyl phosphine in the presence of water.[35] For the preparation of 28-deoxy-28-aminosquamocin (**9**), squamocin (**2**) was treated with three equivalents of triphenyl phosphine and three equivalents of phthalimide in THF in the presence of DEAD to give the 28-mono phthalimide intermediate (again with inversion of absolute configuration at C-28), accompanied by several byproducts (supposedly the other monophthalimides as well as the bis-phthalimide derivatives). The latter was directly mixed with hydrazine to yield the desired 28-deoxy-28-aminosquamocin (**9**).

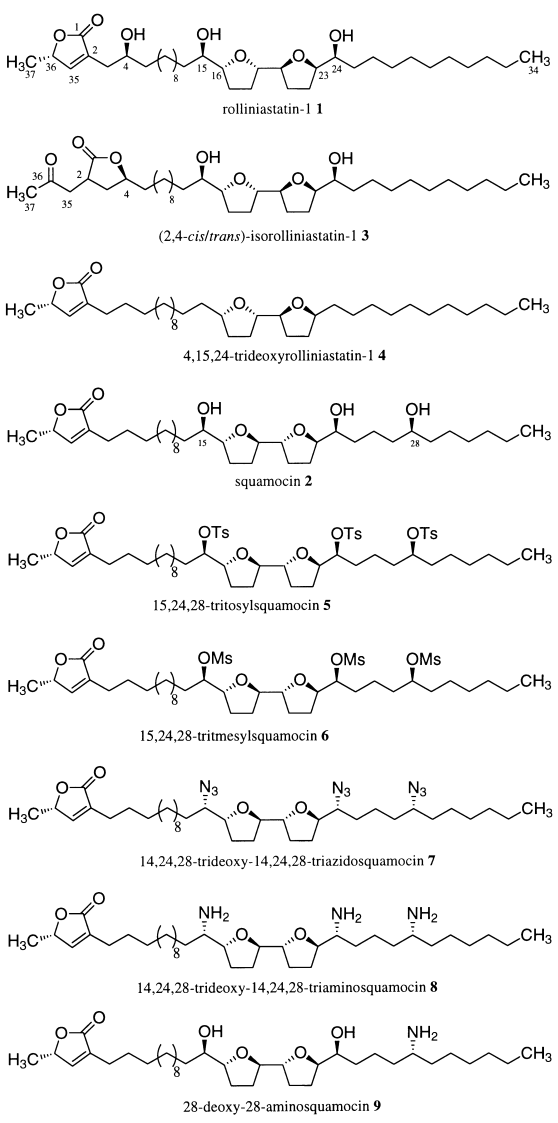
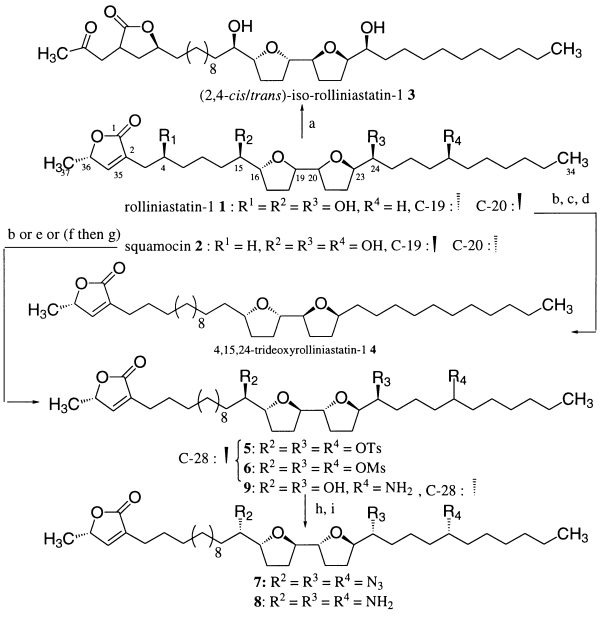


Chart 1.

The cytotoxicity of all nine compounds against human epidermoid carcinoma (KB) and African green monkey (*Cercopithecus aethiops*) kidney epithelial cells (VERO) was evaluated and the results are reported in Table 1. Cytotoxicity is expressed as concentrations of the product which caused 50% growth inhibition (EC_{50}). Assays were performed in 96-well culture plates with threefold 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, 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 acetogenin inducing 50% inhibition of proliferation (EC_{50}) as compared



Scheme 1. Preparation of compounds **3**–**9**. Reagents: (a) Et_2NH, MeOH, 53%; (b) MsCl, pyridine, 70%; (c) NaI, THF; (d) *n*-Bu_3SnH, AIBN, reflux, 17% for the last three steps; (e) TosCl, pyridine, 72%; (f) PPh_3, phthalimide, DEAD, THF, 23%; (g) hydrazine, methanol, 48%; (h) NaN_3, DMSO, 30%; (i) PPh_3, H_2O, THF, 32%.

Table 1. Cytotoxic activity of annonaceous acetogenins and derivatives[a]

Compounds	KB[b,c] EC_{50}	VERO[c,d] EC_{50}
Rolliniastatin-1 (**1**)	$< 1.6\times10^{-7}$	1.1×10^{-2}
(2,4-*cis*/*trans*)-Isorolliniastatin-1 (**3**)	4.8×10^{-5}	2.4×10^{-4}
4,15,24-Trideoxyrolliniastatin-1 (**4**)	3.5×10^{-1}	1.4×10^{-1}
Squamocin (**2**)	1.6×10^{-5}	4.8×10^{-2}
15,24,28-Tritosyl squamocin (**5**)	$< 1.3\times10^{-1}$	$< 1.3\times10^{-1}$
15,24,28-Trimesyl squamocin (**6**)	1.5×10^{-2}	$< 10^{-1}$
15,24,28-Tridoexy-15-24-28-triazido squamocin (**7**)	$< 1.4\times10^{-1}$	$< 1.4\times10^{-1}$
15,24,28-Trideoxy-15,24-28-triamino squamocin (**8**)	2.4×10^{-2}	$< 1.6\times10^{-1}$
28-Deoxy-28-amino squamocin (**9**)	9.7×10^{-2}	$< 1.6\times10^{-1}$
Taxol[a]	1.3	< 11.9
Vinblastine[a]	1.2×10^{-3}	< 3.7

[a] Results are reported in μM; although the modes of action are different, taxol and vinblastine were tested for comparison.
[b] Human epidermoid carcinoma.
[c] When the EC_{50} values were not precisely determined, results are expressed as the limits of the range tested.
[d] African green monkey (*Cercopithecus aethiops*) kidney epithlial cell.

with the control cells were determined from the dose–response curves.

Rolliniastatin-1 (**1**) and squamocin (**2**) have identical molecular formulae ($C_{37}H_{66}O_7$) and only differ by the position of one hydroxyl (located at C-4 and C-28 for rolliniastatin-1 (**1**) and squamocin (**2**), respectively) and inversion of absolute configurations of two of their eight stereogenic centers, located at C-19 and C-20. These differences may be responsible for the remarkable difference in the cytotoxicity toward the KB cells observed for the two compounds (**1** being 100 times more active than **2**). These differences have been observed for several positional isomers of acetogenins. Indeed, it is well accepted that the most active compounds are those substituted at C-4, followed by other substitutions.[3–7] As far as isorolliniastatin-1 (**3**) is concerned, the lower activity for KB cells is also generally observed for the translactonized products, while the increased activity against VERO cells has also been reported.[3–7] In fact, in many cases, the selectivity for various cancer cell lines is very different when iso- and natural parent acetogenins are compared.[3–7,31] More interesting to note is the large decreased activity for trideoxyrolliniastatin-1 (**4**) (KB: EC_{50} 3.5 10^{-1} μM, Table 1). This result proves that the presence of the hydroxyl groups is required for the activity, which can be tentatively correlated to the cation complexation and/or hydrogen bonding abilities of the molecule. In the case of squamocin derivatives (**5**–**9**), it is interesting to note that the tritosyl and triazido compounds **5** and **7** showed decreased activity (KB: EC_{50} 1.3 10^{-1} and 1.4 10^{-1} μM, respectively, Table 1). However, the trimesyl derivative **6** retained some activity (KB: EC_{50} 1.5 10^{-2} μM, Table 1), perhaps because the oxygen atoms of the mesyl group are more accessible than in the case of the tritosyl. For the mono amino and triamino derivatives **9** and **8**, it is noteworthy that some activity remained (KB: EC_{50} 9.7 10^{-2} and 2.4 10^{-2} μM, respectively, Table 1). However, from these results, it is not possible to rule out the influence of the solubility of the different compounds. Indeed, acetogenins are lipophilic, and therefore, must be dissolved in DMSO prior to their use in bioassays. Therefore, when the lipophilic character is increased, as for the sulfonic esters and azido derivative, the low activity may also be explained by low availability to the cells. In order to gain more information, complexation and membrane conformation studies must also be performed with these products.

Finally, the acute in vivo toxicity of both rolliniastatin-1 (**1**) and isorolliniastatin-1 (**3**) after intraperitoneal administration was estimated according to Miller et al.[36] (Table 2).

Mice were treated with acetogenins at different doses, expressed in mg kg^{-1}, in groups of 10 mice per dose. These experiments show that the translactonized product **3** was much less toxic than the parent acetogenin **1** (five mice dead out of 10 when treated with 5 mg kg^{-1} of **1**, whereas 200 mg kg^{-1} are needed for the same result with **3**, Table 2), although their in vitro cytotoxicities were comparable. These results confirm the data previously reported for bullatacin and bullatacinone which only differ from **1** and **3** by the relative configuration of the THF pattern (*threo*/*trans*/*threo*/*trans*/*erythro* for bullatacin and bullatacinone versus *threo*/*cis*/*threo*/*cis*/*erythro* for rolliniastatin-1 and isorolliniastatin-1, respectively).[37] However, these results must be confirmed by repeated experiments. A similar same difference of toxicity was also observed when **1** and **3** were administrered orally (results not shown). Since only small amounts of the amino derivatives were obtained, no in vivo study was possible, but this would

Table 2. Estimation of the acute toxicity of compounds **1** and **3** after intraperitoneal administration[a]

Product	0.675	1.25	2.5	3.125	5	6.25	7.5	10	12.5	20	25	50	100	200
1	0/10	0/110	0/10	—	5/10 (D2)	—	10/10 (D1)	10/10 (D1)	—	10/10 (D1)	—	—	—	—
3	—	—	—	0/10	—	0/10	—	—	0/10	—	0/10	1/10 (D1)	2/10 (D3)	5/10 (D1)

[a] Doses are expressed in mg kg^{-1} on one series of 10 mice (females, 20 g), and the results expressed the number of dead mice out of 10 with delay of observation in days (D); volume injection: 0.1 mL; vehicle: carboxymethyl cellulose.

certainly give complementary information about the bioavailability of these compounds.

In conclusion, these results show for the first time that deoxy compounds lose almost all their in vitro cytotoxic activity, whereas the triamino acetogenin derivatives retain some activity. Furthermore these results are in accordance with those observed for amino-muricatacin[38] and hydroxylamino-derivatives,[39] as well as with other amino bis-THF derivatives.[40] Finally, the very low toxicity observed for isorolliniastatin-1 (**3**) shows that although the natural compounds are usually too toxic, structurally related analogues could be more useful as therapeutic agents. More studies are nevertheless required in order to demonstrate the potency of the amino acetogenins as new specific and efficient anticancer agents.

Experimental

Infra red (IR) spectra were recorded on a Perkin–Elmer 257 apparatus (ν expressed in cm^{-1}). ^1H and ^{13}C NMR spectra were recorded with a Bruker AC-200 (200 MHz) and Bruker AM-400 (400 MHz). Chemical shifts (δ) were expressed in ppm. Coupling constants (*J*) were given in hertz (Hz). Mass spectra (MS) have been recorded on a Nermag-Sidar R10-10C (CIMS) with CH_4 and for the electronic impacts (EIMS) at 40 eV. FABMS-Li spectra were recorded on a Kratos MS-80 Rf. Flash chromatography was performed with silica gel 60 (9385 Merck), silica gel S (31607 Riedel-de-Haën), and silica gel 60H (7736 Merck). TLC was performed on plates coated with silica gel $60F_{254}$ (554 Merck).

Seeds of *A. atemoya* (973 g) were extracted with methanol (4×2 L). After concentration (750 mL), a white precipitate **A** (19.6 g) was obtained by filtration. After concentration of the filtrate, extract **B** (79.7 g) was obtained. After re-dissolution in methanol (250 mL) a liquid/liquid extraction with hexane (3×250 mL) after addition of 2% of water, was performed. The hexanic extract was then evaporated to give an oil (5.2 g, 6.5% of **B**). The methanolic solution was extracted with CH_2Cl_2, to give extract **D** (39.0 g, 48.9% of **B**) after evaporation. **D** (10 g) was purified by flash chromatography starting from toluene:EtOAc:EtOH (30:70:5) v/v/v and ending with a 27:62:11 v/v/v mixture. After preparative HPLC, 23 acetogenins were obtained with rolliniastatin-1 (**1**) (11 mg) and squamocin (**2**) (1.5 g). Seeds of *R. membranacea* were treated as above to give several acetogenins with rolliniastatin-1 (**1**) as the major compound.[41]

(2,4-*cis*/*trans*)-Isorolliniastatin-1 (3). Rolliniastatin-1 (**1**) (210.3 mg from *Rollinia membranacea*) was dissolved in methanol (30 mL) containing 2 mL of diethylamine and stirred at room temperature for 10 h. After evaporation of the methanol at low pressure, the crude product was purified by flash chromatography (toluene:EtOAc, 4:6, v/v) to give isorolliniastatin-1 (**3**) (111 mg). CI MS (CH_4) *m*/*z* 623 $[MH]^+$, 605 $[MH\text{-}H_2O]$, 587 $[MH\text{-}2H_2O]^+$, 569 $[MH\text{-}3H_2O]^+$, 451, 433, 415, 381, 363, 345, 311, 293, 275, 241, 171, 153, 141, 111, 97; ^1H NMR (200 MHz, $CDCl_3$) δ 4.52 (m, H-4 *trans*), 4.36 (m, H-4 *cis*), 3.85 (m, H-16, H-19, H-20, H-23, H-24), 3.45 (m, H-15), 3.15–2.95 (m, H-2 *cis*/*trans*, H-35b *cis*/*trans*), 2.72–2.50 (m, H-3a *cis*, H-35a *cis*/*trans*), 2.20 (s, CH_3-37), 2.10–1.85 (m, H-3a,3b *trans*), 2.00–1.20 (m, CH_2, H-3b *cis*), 0.87 (t, $J = 6.7$ Hz, CH_3-34); ^{13}C NMR (50 MHz, $CDCl_3$) δ 205.5 (C-36), 178.8/178.3 (C-1), 83.0 (C-16 or C-23), 82.9 (C-23 or C-16), 81.1 (C-19 or C-20), 81.0 (C-20 or C-19), 79.3/78.9 (C-4), 44.2/43.7 (C-33), 36.6/34.4 (C-2), 35.3 (C-3), 35.5–22.6 (CH_2), 30.1 (C-37), 14.1 (C-34).

4,15,24-Trideoxyrolliniastatin-1 (4). Rolliniastatin-1 (**1**) (58 mg from seeds of *Rollinia membranacea*) was dissolved in pyridine (4 mL) and mesyl chloride (8.4 mmol, 663 μL) was added. After 1 h of stirring at room temperature, water (20 mL) was added and the organic layer was extracted with 4×10 mL of CH_2Cl_2. The combined organic extracts were washed with a saturated solution of $CuSO_4$ followed by water and finally dried over Na_2SO_4. CH_2Cl_2 was then evaporated, and the crude product purified by flash chromatography with EtOAc:hexane (6:4, v/v). 4,15,24-Trimesylrolliniastatin-1 (37 mg) was thus obtained. ^1H NMR (200 MHz, $CDCl_3$) δ 7.27 (d, $J = 1.1$ Hz, H-35), 5.05 (dq, $J = 6.9$, 1.4 Hz, H-36), 4.90 (q, $J = 6.3$, H-4), 4.78 (m, H-24), 4.52 (q, $J = 6.3$ Hz, H-15), 3.96 (m, H-16, H-23), 3.81 (m, H-19, H-20), 3.17 (s, SO_2CH_3-24), 3.05 (s, SO_2CH_3-15), 2.99 (s, SO_2CH_3-4), 2.68 (m, H-3), 2.00–1.20 (m, CH_2), 1.44 (d, $J = 6.7$ Hz, CH_3-37), 0.87 (t, $J = 6.7$ Hz, CH_3-34).

The latter (33 mg, 38.5 μmol) was dissolved in THF (5 mL) and NaI (86.7 mg, 0.58 mmol, 15 equiv) was added. The mixture was heated under reflux for 10 h. Then tributyltin hydride (174.2 mg, 161 μL, 0.60 mmol) and a catalytic amount of azo-bis-isobutyronitrile (AIBN) were added after cooling. The mixture was then heated under reflux for 6 h with stirring. After cooling, water (20 mL) was added and the organic layer extracted 3×10 mL of CH_2Cl_2. The combined organic extracts were washed with water and then dried over Na_2SO_4. After purification by preparative thin layer

chromatography (toluene:EtOAc, 5:1, v/v; R_f 0.52) 4,15,24-tridesoxyrolliniastatin-1 (**4**) (4 mg, 17%) was obtained. CI MS (CH_4) *m/z* 592 $[MNH_4]^+$, 575 $[MH]^+$, 279; EI MS *m/z* 556, 349 (100), 269, 251, 235, 209, 195, 181, 167, 153, 139, 125, 111, 97; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.98 (d, J = 1.5 Hz, H-35), 4.99 (dq, J = 6.8, 1.7 Hz, H-36), 3.80 (m, H-16, H-19, H-20, H-23), 2.26 (t, J = 7.7 Hz, H-3), 2.10–1.20 (m, CH_2), 1.40 (d, J = 6.8 Hz, CH_3-37), 0.88 (t, J = 6.9 Hz, CH_3-34).

15,24,28-Tritosylsquamocin (5). Squamocin (**2**) (15 mg, 24.1 μmol) was dissolved in pyridine (1 mL) at 0°C and tosyl chloride (100 mg, 0.52 mmol, 21.8 equiv) was added and the solution stirred for 12 h at room temperature. Water (2 mL) was added and the organic layer was extracted with 5×2 mL of $CHCl_3$. The combined organic extracts were washed with HCl 0.1 M then water and dried over Na_2SO_4. The crude mixture was then purified by flash chromatography with a gradient of solvent (CH_2Cl_2 100% to EtOAc 100%) to give the desired product **5** (19 mg, 72%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.85–7.27 (2m, 12H), 6.99 (d, J = 1.5 Hz, H-35), 4.99 (dq, J = 6.7, 1.6 Hz, H-36), 4.50 (m, H-15, H-24, H-28), 4.02 (m, H-16 or H-23), 3.88 (m, H-23 or H-16), 3.72 (m, H-19 or H-20), 3.45 (m, H-20 or H-19), 2.44–2.43 (2s, 9H), 2.26 (t, J = 7.0 Hz, H-3), 2.00–1.20 (m, CH_2), 1.40 (d, J = 6.9 Hz, CH_3-37), 0.85 (t, J = 6.8 Hz, CH_3-34).

15,24,28-Trimesylsquamocin (6). Squamocin (**2**) (60 mg, 96.5 μmol) was dissolved in pyridine (2.5 mL) at 0°C and mesyl chloride (255 μL, 3.29 mmol, 34 equiv) was added and the temperature allowed to reach 20°C. After 20 h of stirring, water (20 mL) was added and the organic layer extracted with 5×10 mL of $CHCl_3$. The combined organic extracts were washed with HCl 0.1 M, followed by water then dried over Na_2SO_4. After $CHCl_3$ evaporation, the crude material was purified by flash chromatography (CH_2Cl_2 100% then EtOAc 100%) to afford the desired product **6** (58 mg, 70%). CI MS (CH_4) *m/z* 761 $[MH\text{-}CH_3SO_3H]^+$, 665 $[MH\text{-}2CH_3SO_3H]^+$, 569 $[MH\text{-}3CH_3SO_3H]^+$, 551, 193, 97; EI MS *m/z* 760, 675, 664, 579, 568, 416, 373, 347, 193, 97, 79; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.98 (d, J = 1.5 Hz, H-35), 4.98 (dq, J = 8.0, 1.7 Hz, H-36), 4.73 (m, H-24, H-28), 4.50 (m, H-15), 4.03 (m, H-16, H-23), 3.85 (m, H-19, H-20), 3.04 (s, CH_3SO_2), 3.00 (s, CH_3SO_2), 2.99 (s, CH_3SO_2), 2.24 (t, J = 7.0 Hz, H-3), 2.00–1.20 (m, CH_2), 1.39 (d, J = 6.8 Hz, CH_3-37), 0.87 (t, J = 6.8 Hz, CH_3-34).

15,24,28-Trideoxy-15,24,28-triazidosquamocin (7). Sodium azide (190 mg, 2.92 mmol, 13 equiv/mesyl function) was dissolved in DMSO (4 mL) and a DMSO solution of **6** (58 mg, 75.3 μmol, in 1 mL) was added. After stirring at room temperature for 12 h, water (20 mL) was added. Organic layer was then extracted with 5×10 mL of $CHCl_3$. The combined organic extracts were then washed with water, dried over Na_2SO_4 and finally the solvents were evaporated under reduced pressure. The crude reaction mixture was analyzed by ^{1}H NMR and showed appearance of a single azido group (located at C-28). The crude mixture was thus re-dissolved in 2 mL of DMSO, a large excess of NaN_3 (200 mg) was added and the solution was heated under reflux for 20 h. After cooling, an identical work up gave 49 mg of a crude product, which was purified by flash chromatography (CH_2Cl_2:EtOAc, 100:1, v/v) to yield **7** (14 mg, 30%). CI MS (CH_4) *m/z* 726 $[MC_2H_5]^+$, 698 $[MH]^+$, 670, 655, 627, 613, 584, 460, 442, 432, 377, 349, 334, 294; EI MS *m/z* 584, 460, 432, 377, 362, 334, 320, 306, 294, 251, 237, 208, 194, 167, 111, 97; IR (film) ν_{max} cm^{-1}: 2103, 1756; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.98 (d, J = 1.4 Hz, H-35), 4.99 (dq, J = 7.0, 1.5 Hz, H-36), 4.00 (m, H-16, H-19, H-20, H-23), 3.57 (m, H-15), 3.24 (m, H-28), 3.13 (m, H-24), 2.26 (t, J = 7.0 Hz, H-3), 2.00–1.20 (m, CH_2), 1.40 (d, J = 6.7 Hz, CH_3-37), 0.89 (t, J = 6.7 Hz, CH_3-34).

15,24,28-Trideoxy-15,24,28-triaminosquamocin (8). Compound **7** (8 mg) was dissolved in THF (0.8 mL), and triphenylphosphine (50.8 mg, 1.7 equiv) and water (15 μL) were added. After stirring at room temperature for 18 h, THF was evaporated off and the crude mixture dissolved in benzene (3 mL). HCl 0.01 M was added and organic layer separated. The aqueous layer was then treated with a 1% solution of $NaHCO_3$, and extracted with ethyl ether. Benzenic and ether solutions, both presenting a positive reaction both with Kedde reagent and ninhydrin, were combined and evaporated. The crude mixture was purified by chromatography on Sephadex® LH-20 (Pharmacia, MeOH 100%) and then by preparative TLC (EtOH:H_2O:AcOH, 4:5:1, v/v/v) to afford **8** (2.3 mg, 32%). CI MS (CH_4) *m/z* 620 $[MH]^+$, 602, 534, 517, 434, 325, 307, 294, 278, 255, 201, 185, 168, 151, 97; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.00 (d, J = 1.4 Hz, H-35), 4.99 (dq, J = 6.3, 1.3 Hz, H-36), 4.00–3.60 (m, H-16, H-19, H-20, H-23), 3.20–2,80 (m, H-15, H-24, H-28), 2.26 (t, J = 7.0 Hz, H-3), 2.00–1.20 (m, CH_2), 1.41 (d, J = 6.8 Hz, CH_3-37), 0.88 (t, J = 6.8 Hz, CH_3-34).

28-Deoxy-28-aminosquamocin (9). Squamocin (**2**) (50 mg, 80.4 μmol) and triphenylphosphine (72 mg, 3.4 equiv) with phthalimide (40 mg, 0.27 mmol, 3.4 equiv) were dissolved in THF (3 mL). Then diethylazodicarboxylate (43 μL, 0.27 mmol, 3.4 equiv) was added at room temperature. After stirring overnight, the solvent was evaporated and the crude mixture dissolved in ethyl ether and the solid filtered. After evaporation of the solvent under reduced pressure, the crude mixture was purified by flash chromatography (EtOAc:toluene, 5:5, v/v), to afford 14 mg of 28-phthalimidosquamocin (23%). CI MS (CH_4) *m/z* 780 $[MC_2H_5]^+$, 752 $[MH]^+$, 734 $[MH\text{-}H_2O]^+$; 716 $[MH\text{-}2H_2O]^+$, 698, 622, 604, 486, 456, 436, 347, 295, 169, 148, 97; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.80 (m, 4H), 6.98 (d, J = 1.3 Hz, H-35), 5.00 (dq, J = 6.8, 1.8 Hz, H-36), 4.18 (m, H-28), 3.80 (m, H-16, H-19, H-20, H-23, H-24), 3.38 (m, H-15), 2.26 (t, J = 7.0 Hz, H-3), 2.00–1.20 (m, CH_2), 1.40 (d, J = 6.8 Hz, CH_3-37), 0.83 (t, J = 6.8 Hz, CH_3-34).

The latter (10 mg) was then dissolved in methanol (3 mL) and hydrazine (10 μL, 8.8 mg) was added and the solution heated under reflux. After 27 h, the methanol was evaporated and the crude reaction mixture purified by flash chromatography (EtOAc then EtOAc:MeOH:

NH_4OH, 100:10:5, v/v/v). **9** was thus obtained (4 mg, 48%). CI MS (CH_4) *m/z* 650 $[MC_2H_5]^+$, 622 $[MH]^+$, 604 $[MH\text{-}H_2O]^+$; 594, 578, 536, 356, 347, 326, 309, 295, 256, 186, 168, 114, 97; ^1H NMR (200 MHz, $CDCl_3$) δ6.98 (d, $J = 1.5$ Hz, H-35), 4.99 (dq, $J = 6.7$, 1.6 Hz, H-36), 3.86 (m, H-16, H-19, H-20, H-23, H-24), 3.39 (m, H-15), 2.72 (m, H-28), 2.26 (t, $J = 7.3$ Hz, H-3), 2.00–1.20 (m, CH_2), 1.40 (d, $J = 6.8$ Hz, CH_3-37), 0.88 (t, $J = 6.8$ Hz, CH_3-34).

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