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# Semisynthesis and biological activity of aminoacyl triesters of squamocin, an annonaceous acetogenin

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**Abstract**—A number of aminoacyl triesters of squamocin **1**, a cytotoxic acetogenin isolated from the seeds of *Annona reticulata*, have been synthesized in two to three steps from protected (L)-aminoacids and squamocin **1** using standard coupling/deprotection procedures. These semisynthetic analogs were tested on submitochondrial particles (SMP) for their complex I inhibitory activities, and against KB 3-1 cells in vitro. All triesters derivatives exhibited a complete extinction of activity at the enzymatic level, correlated to a reduced though modulated cytotoxicity in comparison with squamocin **1**. This activity can apparently be considered as a function of the amphipathy of the analogs, the more amphiphilic ones being the more cytotoxic.


## 1. Introduction

Annonaceous acetogenins exhibit a broad range of biological activities (cytotoxic, antiparasitic, insecticide...)[1–3] as a result of the inhibition of the mitochondrial NADH-ubiquinone oxidoreductase (complex I),[4,5] causing a decrease of ATP biosynthesis and cell death by apoptotic mechanisms.[6,7] In addition to their often spectacular cytotoxicity against various sensitive and MDR expressing cancer cell lines in vitro and in vivo,[5,8–12] annonaceous acetogenins appear as potential antitumor agents because of their selective inhibition of the plasmic NADH oxidoreductase overexpressed and deregulated in transformed cells.[13] From a mechanistic point of view, the precise interaction between these new type of inhibitors and their huge mitochondrial target remains unknown. Two observations were the basis of the present study: (a) the transformation of the secondary alcohols of acetogenins into acetate or mesyl functions is known to systematically lead to a disappearance of cytotoxicity;[1,9] (b) such acylated or sulfonylated derivatives can retain a high degree of enzymatic inhibitory activity.[14] This ambiguity led us to investigate whether acylation of these hydroxy groups by moieties containing electron-rich functions (amino, imidazole, hydroxymethyl, phenol...) could maintain the native molecule activities by acting even at some distance from the aliphatic backbone. α-Aminoacyl derivatives of a representative acetogenin correspond to ideal candidates for such a study, since selective deprotection of the acyl residues allows access to diverse protic analogs of the lead inhibitor. Moreover, a very significant enhancement of the hydrosolubility of such derivatives can be expected. The chosen acetogenin to be modified was squamocin **1** (Fig. 1), a highly cytotoxic agent and potent respiratory inhibitor among this class of natural products.[9,14–17]

## 2. Results and discussion

### 2.1. Semisynthesis

The Phe, Trp, and Pro α-aminoacids were selected to evaluate the influence of the α-amino function on the biological activity of triester squamocin derivatives. Short chain polar α-aminoacids such as Ser and His were selected to evaluate the influence of proximal protic functions. On the other hand, considering that the behavior of membrane partition of natural acetogenins might be a key feature for their bioactivity,[18,19] the creation of hydrophilic distal functions was targeted by

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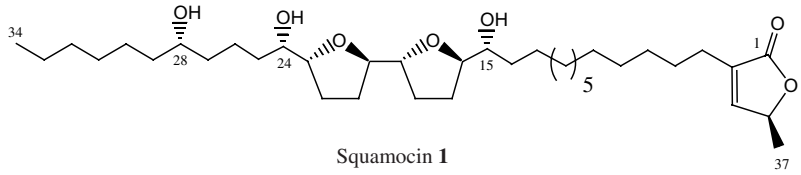



Squamocin **1**

**Figure 1.**

selecting the Glu, Tyr, and Lys α-aminoacids for the *O*-acylation of squamocin **1**. The desired α-aminoacyl triesters of squamocin **1** were semisynthesized in two steps using procedures from peptide chemistry. The native acetogenin was first esterified with good yields in presence of an excess $N_{\alpha}$-BOC protected (L)-α-aminoacids, using DCC[†] and a catalytic amount of 4-DMAP (Scheme 1).

The α-amino groups of the Phe, Trp, and Lys and Pro squamocin triesters **2p**–**5p** were deprotected by use of HF in pyridine (Scheme 2). In order to prevent epimerization of the sensitive lactonic C-36 stereocenter, known to readily occur in polar protic media in the presence of a weak base,[20,21] the corresponding ammonium fluorides **2**, **3**, and **4a** were purified without being turned into their corresponding free bases. On the other hand, the trifluoride salt of Pro squamocin triester **5** could not be separated from residual pyridinium fluoride because of its complete solubility in aqueous phase, similarly to the trifluoride salt of Trp squamocin triester **3**. Compound **5** was therefore obtained by treating **5p** with trifluoroacetic acid and purified by chromatography over Sephadex® LH-20.

The lateral chain functions of several triesters were deprotected using the following reagents, with acceptable yields (Scheme 3): (a) Pd–C (10%)/1,4-cyclohexadiene system in hot absolute ethanol,[22,23] allowing a smooth catalytic hydrogenolysis of the *O*-benzylether groups of the Ser and Tyr triesters **6p** and **7p**, and of the $N_{\varepsilon}$-benzyloxycarbonyl and *O*-benzylester groups of the Lys and Glu triesters **4p** and **8p**, respectively. In no case, reduction of the terminal α,β-unsaturated lactone was observed; (b) excess HOBT in THF for the detosylation of the imidazole nuclei[24] of the $N_{im}$-Ts-His triester **9p**.

### 2.2. Biological evaluation

A selection of squamocin analogs was tested against complex I from beef heart SMP, and their inhibitory activities evaluated (Table 1). Compounds **2p**, **2**, and **5** exhibited a complete absence of potency in comparison with squamocin **1**, its triacetylated derivative **1a** and rotenone, but were significantly more active than previously described tri-*O*-TBDMS squamocin **1s**.[25] Moreover, it has to be noticed the similar activities of the tri-$N_{\alpha}$-BOC protected derivative **2p** and its corresponding triamino analog **2**. This fact could reflect the probable enzymatic interaction of the polyoxygenated domain of bioactive acetogenins through its electron-donating character, suggested by the strong inhibitory activities of non-hydroxylated, acetylated or carbonylated acetogenins.[14]

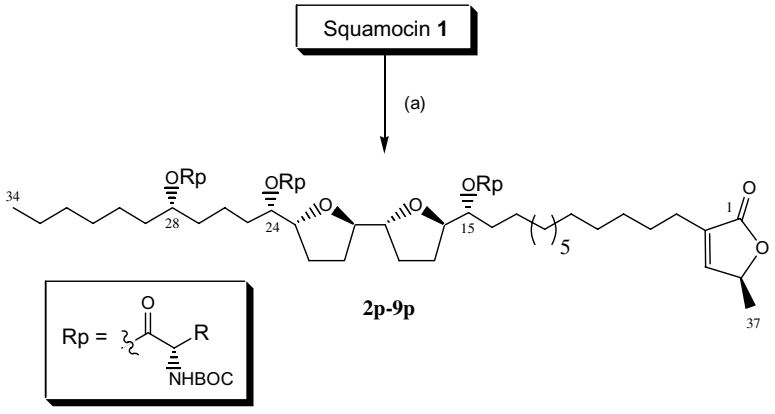
All fully or partially deprotected squamocin triesters were tested against KB 3-1 cells in vitro (human epidermoid nasopharyngeal carcinoma), and their cytotoxicity evaluated in comparison with tri-*O*-TBDMS squamocin **1s** tested as an inactive and metabolically inert derivative of the parent molecule[25] (Table 2).

In spite of similar or enhanced polarity in comparison with the natural acetogenin, all compounds exhibited strongly reduced cytotoxicities relatively to squamocin **1**, but were 10–1000 times more active than its trisilyl ether **1p** in most cases. The most amphipathic triesters, possessing free α-amino groups (as their ammonium) appeared to be the more cytotoxic (Pro, Phe, $N_{\varepsilon}$-(*Z*)-Lys and Trp derivatives), followed by some $N_{\alpha}$-BOC compounds with a deprotected lateral chain (Ser, His, and Lys derivatives). The location of protic functions was found to be an important factor for the activity: apart from the amphiphilic character, it seemed that the restoration of a polar environment close to the aliphatic chain of the acetogenin could account for the residual cytotoxic activities of α-amino analogs. Thus, the Ser triester **6** was 10–30 times less cytotoxic than the last derivatives, despite the existence of three alcohol functions similarly to squamocin **1**. Identically, the Lys triester **4b** exhibited a twenty times lower activity than its analog **4a**. The presence of three hydrophilic carboxylic acid (triester **8**) or imidazole moieties (triester **9**) did not compensate for the peracylation of the hydroxyles of squamocin **1**, these analogues being weakly or not cytotoxic. Similarly, the rigid triphenolic derivative **7**, bearing distant protic functions, was devoid of activity.

## 3. Conclusion

A number of aminoacyl triesters of squamocin **1**, a cytotoxic annonaceous acetogenin, have been semisynthesized. Despite the promising hydrosolubilities of most analogs, the derivatization of the secondary alcohols of squamocin **1** resulted in a dramatic loss of activity, although these triesters were cytotoxic relatively to the inactive lipophilic triether **1p**. As previously suggested, our results show that amphipathy is a key factor for the bioactivity of acetogenin-derived inhibitors, taking into account that molecules possessing a terminal α,β-

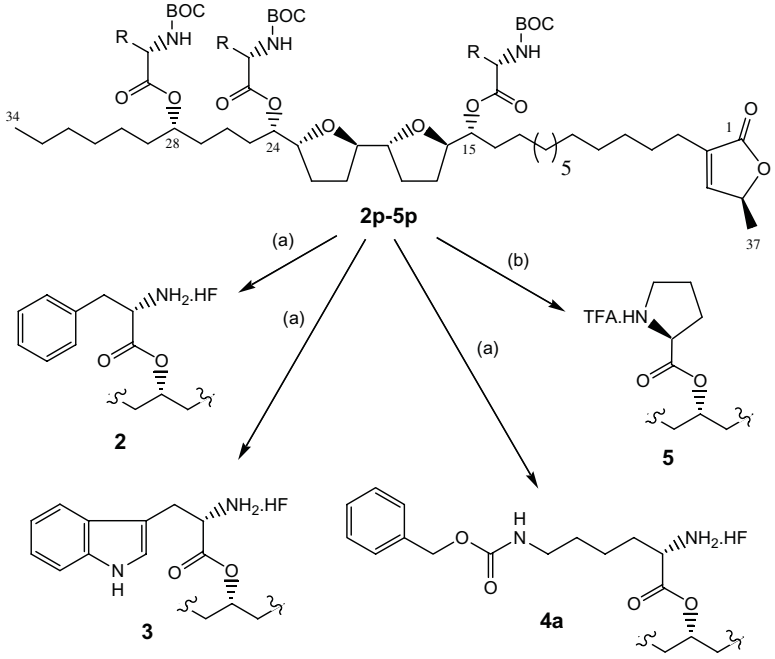
[†]Abbreviations: DCC: dicyclocarbodiimide; 4-DMAP: 4-dimethylaminopyridine; HOBT: *N*-hydroxybenzotriazole; SMP: submitochondrial particles; THF: tetrahydrofurane.




(R = protected lateral chain)

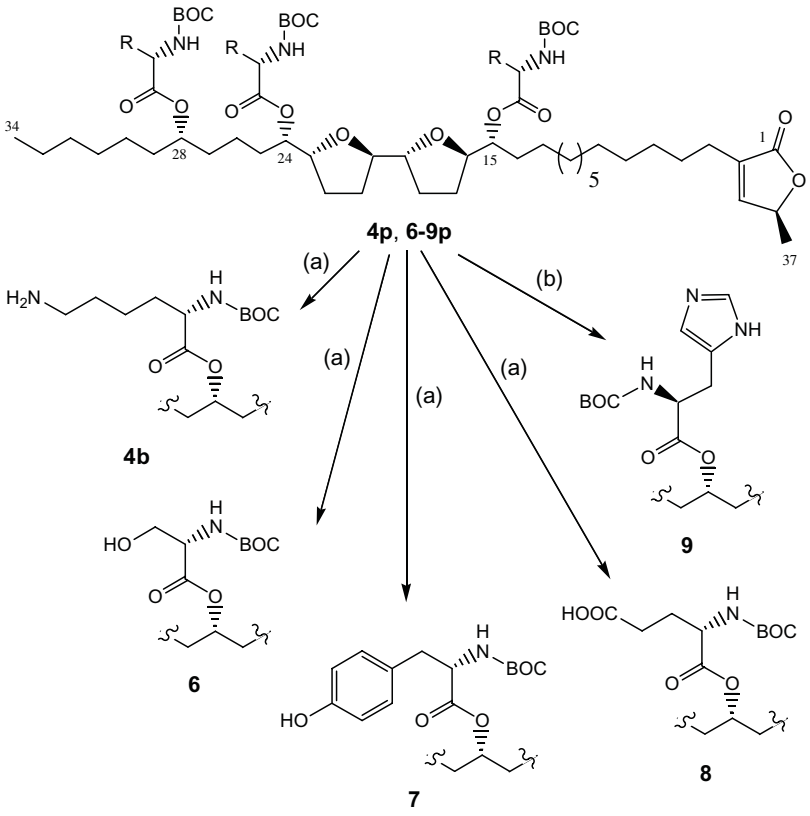
| RpOH | Squamocin triesters | Yield % |
|---|---|---|
| $N_\alpha$-(BOC)-PheOH | **2p** | 86 |
| $N_\alpha$-BOC-TrpOH | **3p** | 73 |
| $N_\alpha$-(BOC)-$N_\varepsilon$-(*Z*)-LysOH | **4p** | 83 |
| $N_\alpha$-(BOC)-ProOH | **5p** | 71 |
| $N_\alpha$-(BOC)-*O*-(Bn)-SerOH | **6p** | 81 |
| $N_\alpha$-(BOC)-*O*-(Bn)-TyrOH | **7p** | 76 |
| $N_\alpha$-(BOC)-*O*-(Bn)-GluOH | **8p** | 68 |
| $N_\alpha$-(BOC)-$N_{im}$-(Ts)-HisOH | **9p** | 37 |

**Scheme 1.** Semisynthesis of the protected 15,24,28-tri-$N_\alpha$-BOC-(aminoacyl)-squamocin derivatives **2p**–**9p**. Reagents and conditions: (a) squamocin **1**, $R_P$OH, DCC, cat. 4-DMAP, EtOAc or $CH_2Cl_2$, 0 °C to rt.




**Scheme 2.** Deprotection of the $N_\alpha$-BOC groups of the triesters **2p**–**5p**. Reagents and conditions: (a) **2p**–**4p**, HF/Py, anisole, 0 °C to rt, 2 h (68% **2**, 39% **3**, 57% **4a**); (b) **5p**, TFA, $CH_2Cl_2$, 0 °C, 2 h (55%).

unsaturated lactone but devoid of amphiphily (γ-methylbutenolides substituted by long aliphatic chains, some type E acetogenins, squamocin triether **1p**) are significantly less active on mitochondrial complex I than most of the obtained triesters.[26,27] Beside, the restoration of a protic environment as close as possible to the aliphatic skeleton of the acetogenin seems to be responsible for the superior cytotoxicity of certain squamocin triesters. However, this activity appears to be poorly specific in term of enzymatic inhibition, and the possibility of a




**Scheme 3.** Deprotection of the lateral chains of triesters **4p** and **6p**–**9p**. Reagents and conditions: (a) **4p**, **6**–**8p**, Pd–C (10%), 1,4-cyclohexadiene, EtOH, 40–65 °C, 2–8 h (86% **4b**, 52% **6**, 60% **7**, 32% **8**); (b) **9p**, HOBT, THF, rt, 5 h (42%).

**Table 1.** Complex I inhibitory activities ($IC_{50}$ nM) of squamocin triesters **2p**, **2**, and **5**

| Compound | NADH oxydase[a] |
|---|---|
| Squamocin **1** | 0.8 ± 0.09 |
| Tri-(ProNH.TFA)-squamocin **5** | 247.7 ± 20.5 |
| Tri-(PheNH$_2$.HF)-squamocin **2** | 440.0 ± 42.4 |
| Tri-($N_\alpha$-BOC-Phe)-squamocin **2p** | 625.0 ± 92.9 |
| Tri-(*O*-Ac)-squamocin **1a**[b] | 5.0 ± 1.3 |
| Tri-(*O*-TBDMS)-squamocin **1s**[b] | >5000 |
| Rotenone[b] | 5.4 ± 0.6 |

[a] Tests performed on bovine heart submitochondrial particles (SMP).
[b] Reference compounds.

**Table 2.** Cytotoxic activities ($IC_{50}$ nM) of the squamocin triesters **2**–**9**

| Compound | KB 3-1[a] |
|---|---|
| Squamocin **1** | $2.6 \times 10^{-14}$ |
| Tri-(ProNH.TFA)-squamocin **5** | $2.0 \times 10^{-8}$ |
| Tri-(PheNH$_2$.HF)-squamocin **2** | $6.1 \times 10^{-8}$ |
| Tri-($N_\varepsilon$-*Z*-LysNH$_2$.HF)-squamocin **4a** | $6.3 \times 10^{-8}$ |
| Tri-(TrpNH$_2$.HF)-squamocin **3** | $2.4 \times 10^{-7}$ |
| Tri-($N_\alpha$-BOC-SerOH)-squamocin **6** | $5.5 \times 10^{-7}$ |
| Tri-($N_\alpha$-BOC-LysNH$_2$)-squamocin **4b** | $1.1 \times 10^{-6}$ |
| Tri-($N_\alpha$-BOC-HisNH)-squamocin **9** | $1.7 \times 10^{-6}$ |
| Tri-($N_\alpha$-BOC-TyrOH)-squamocin **7** | $>10^{-5}$ |
| Tri-($N_\alpha$-BOC-GluCOOH)-squamocin **8** | $>10^{-5}$ |
| Tri-(*O*-TBDMS)-squamocin[b] **1p** | $>10^{-5}$ |
| NaF[b] | $5.3 \times 10^{-3}$ |
| Paclitaxel[c] | $2.1 \times 10^{-11}$ |

[a] KB 3-1: human nasopharyngeal epidermoïd carcinoma.
[b] Control compounds.
[c] Reference compound.

specific membrane perturbation by highly amphiphilic, positively charged lipophilic compounds,[28,29] at a cellular or mitochondrial level, cannot be excluded. Based on partition studies, Shimada et al. proposed that the polar domain of acetogenins locates at the water–lipids interface of the inner mitochondrial membrane, allowing the specific interaction of their terminal lactone with complex I.[18,19] Considering the increased hydrophilicity of the central core of most semisynthesized triesters (i.e., their superior anchorage potential) but their dramatic loss of activity, our results are not in favor of this modeling and suggest a specific interaction between the polyoxygenated domain of annonaceous acetogenins and mitochondrial complex I.

## 4. Experimental

NMR spectra were recorded on Bruker AC-200 (200 MHz) or Bruker AM-400 (400 MHz) spectrometers. Mass spectra (MS or HRMS) were recorded on Kratos MS-80 Rf. Optical rotations were measured on a Schmidt–Haensch polarimeter E at 589 nm. Column chromatography was performed with silica gel 60 (9385 Merck), Florisil® (Merck), Sephadex® LH-20

(Pharmacia), or alumina 90 standard II–III (1097 Merck). TLC was performed on aluminum plates coated with silica gel $60F_{254}$ (554 Merck) and revealed with sulfuric vanillin reagent. Solvents used in this study were simply redistilled before use.

### 4.1. Extraction

Squamocin **1** was isolated in significant quantity from the seeds of *Annona reticulata*, collected in Viet-Nam, using a described procedure.[30] Its chemical identity was determined by extensive $^1H$ and $^{13}C$ NMR (including HOHAHA, HMQC, and HMBC) experiments, and comparison with an authentic sample previously isolated from *Annona cherimolia*.[9]

### 4.2. Semisynthesis

**4.2.1. 15,24,28-Tri-*O*-($N_\alpha$-BOC-phenylalanyl)-squamocin 2p.** To a solution of 120 mg (0.193 mmol) squamocin **1** in 2.5 mL EtOAc were added 256 mg (0.965 mmol) $N_\alpha$-BOC-phenylalanine and catalytic 4-DMAP. The obtained mixture was cooled to 0 °C and a solution of 199 mg (0.965 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction medium was brought back to room temperature and stirred 15 h, then evaporated under reduced pressure. The residue was retaken in cyclohexane (3 × 5 mL) and filtered on Whatman GF/A. The crude product was chromatographed over a column of silica gel (EtOAc/cyclohexane 30:70 v/v), furnishing 226 mg (86%) triester **2p** as a colorless resin. $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 0.87 (t, 3H, H-34, $J$ = 6.8 Hz), 1.24 (m, 2H, H-26), 1.38 (s, 27H, $^t$Bu–O), 1.40 (d, 3H, H-37, $J$ = 6.8 Hz), 1.46 (m, 4H, H-27, H-29), 1.53 (m, 4H, H-4, H-14), 1.74 (m, 4H, H-18, H-21), 1.88 (m, 4H, H-17, H-22), 2.25 (t, 2H, H-3, $J$ = 7.1 Hz), 2.99 (m, 3 H, $H_\beta$), 3.14 (m, 3H, $H_\beta$), 3.86 (m, 2H, H-19, H-20), 3.96 (m, 2H, H-16, H-23), 4.55 (m, 3H, $H_\alpha$), 4.82 (m, 1H, H-28), 4.85 (m, 1H, H-24), 4.97 (m, 4H, H-15, $NH_{BOC}$), 4.99 (dq, 1H, H-36, $J$ = 1.3 Hz, $J$ = 6.8 Hz), 6.98 (d, 1H, H-35, $J$ = 1.3 Hz), 7.15–7.29 (m, 15H, H-arom.). ESIMS $m/z$ 1388 $[M+Na]^+$. $[\alpha_D]$ +28 ($c$ 1, $CHCl_3$).

**4.2.2. 15,24,28-Tri-*O*-(phenylalanyl)-squamocin tri-(ammonium fluoride) 2.** A solution of 52 mg (38.1 μmol) triester **2p** in 0.5 mL anisole was cooled to 0 °C and 0.5 mL of a 65% HF solution in pyridine was added dropwise. The reaction medium was stirred at that temperature for 2 h, then diluted by EtOAc (10 mL) and treated by water (5 mL). The organic phase was decanted and washed by water (3 × 3 mL), dried ($Na_2SO_4$), filtered, and evaporated under reduced pressure. The crude product was chromatographed over a column of Florisil® (EtOAc/MeOH 90:10 v/v), furnishing 29 mg (68%) triester **2** as a white powder. $^1H$ NMR (MeOD, 400 MHz) $\delta$ 0.88 (t, 3H, H-34, $J$ = 6.8 Hz), 1.25 (m, 2H, H-26), 1.36 (d, 3H, H-37, $J$ = 6.8 Hz), 1.48 (m, 2H, H-14), 1.53 (m, 2H, H-4), 1.67 (m, 4H, H-18, H-21), 1.94 (m, 4H, H-17, H-22), 2.22 (t, 2H, H-3, $J$ = 7.3 Hz), 2.89 (m, 3H, $H_\beta$), 3.03 (m, 3H, $H_\beta$), 3.71 (m, 3H, $H_\alpha$), 3.82 (m, 2H, H-19, H-20), 3.97 (m, 2H, H-16, H-23), 4.83 (m, 1H, H-24), 4.86 (m, 1H, H-28), 4.92 (m, 4H, H-15, $NH_2$), 5.04 (dq, 1H, H-36, $J$ = 1.5 Hz, $J$ = 6.8 Hz), 7.17–7.29 (m, 16H, H-35, H-arom.). ESIMS $m/z$ 1064 $[M+H]^+$, 1086 $[M+Na]^+$.

**4.2.3. 15,24,28-Tri-*O*-($N_\alpha$-BOC-tryptophoyl)-squamocin 3p.** To a solution of 104 mg (0.167 mmol) squamocin **1** in 2 mL EtOAc were added 254 mg (0.836 mmol) $N_\alpha$-BOC-tryptophane and catalytic 4-DMAP. The mixture was cooled to 0 °C and a solution of 172 mg (0.836 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction media was brought back to room temperature and stirred for 15 h, then evaporated under reduced pressure. The residue was retaken in ether (50 mL) and filtered, and the filtrate washed by water (3 × 5 mL), dried ($Na_2SO_4$), and filtered. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 50:50 v/v), furnishing 181 mg (73%) triester **3p** as an amorphous white solid. $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 0.86 (t, 3H, H-34, $J$ = 6.8 Hz), 1.20 (m, 2H, H-26), 1.35 (m, 2H, H-14), 1.40 (d, 3H, H-37, $J$ = 6.8 Hz), 1.43 (s, 27H, $^t$Bu–O), 1.49 (m, 4H, H-27, H-29), 1.54 (m, 4H, H-4, H-25), 1.61 (m, 4H, H-18, H-21), 1.84 (m, 4H, H-17, H-22), 2.26 (t, 2H, H-3, $J$ = 7.6 Hz), 3.23 (m, 3H, $H_\beta$), 3.30 (m, 3H, $H_\beta$), 3.80 (m, 2H, H-19, H-20), 3.93 (m, 2H, H-16, H-23), 4.65 (m, 3H, $H_\alpha$), 4.89 (m, 1H, H-24), 4.98 (m, 2H, H-15, H-28), 5.0 (dq, 1H, H-36, $J$ = 1,5 Hz, $J$ = 6.8 Hz), 5.04 (m, 3H, $NH_{BOC}$), 6.89–7.07 (m, 3H, H-2′), 6.98 (d, 1H, H-35, $J$ = 1.5 Hz), 7.0–7.59 (m, 13H, H-35, H-arom.), 8.51–8.73 (m, 3H, $NH_{ind}$). ESIMS $m/z$ 1505 $[M+Na]^+$, 764 $[M+2Na]^{2+}$. $[\alpha]_D$ −10 ($c$ 1, $CHCl_3$).

**4.2.4. 15,24,28-Tri-*O*-(tryptophoyl)-squamocin tri-(ammonium fluoride) 3.** A solution of 40 mg (27.0 μmol) triester **3p** in 0.4 mL anisole was cooled to 0 °C and 0.4 mL of a 65% HF solution in pyridine was added dropwise. The reaction medium was stirred at that temperature for 2 h, then diluted by EtOAc (20 mL) and treated by water (8 mL). The organic phase was decanted and washed by water (3 × 5 mL), dried ($Na_2SO_4$), filtered, and evaporated under reduced pressure. The crude product was chromatographed over a column of Florisil® (EtOAc/MeOH 80:20 v/v), furnishing 13 mg (39%) triester **3** as a white powder. $^1H$ NMR (MeOD, 400 MHz) $\delta$ 0.93 (t, 3H, H-34, $J$ = 6.8 Hz), 1.33 (d, 3H, H-37, $J$ = 6.8 Hz), 1.73 (m, 4H, H-18, H-21), 1.81 (m, 4H, H-17, H-22), 2.26 (t, 2H, H-3, $J$ = 7.8 Hz), 3.09 (m, 3H, $H_\beta$), 3.25 (m, 3H, $H_\beta$), 3.75 (m, 3H, $H_\alpha$), 3.84 (m, 2H, H-19, H-20), 3.92 (m, 2H, H-16, H-23), 4.63–4.95 (m, 3H, H-15, H-24, H-28), 5.06 (dq, 1H, H-36, $J$ = 1.3 Hz, $J$ = 6.8 Hz), 6.96–7.63 (m, 16H, H-35, H-arom.). ESIMS $m/z$ 1183 $[M+H]^+$. HRESIMS $m/z$ 1180.7180 (calcd for $C_{70}H_{96}N_6O_{10}$: 1180.7188).

**4.2.5. 15,24,28-Tri-*O*-($N_\alpha$-BOC-$N_\varepsilon$-*Z*-lysyl)-squamocin 4p.** To a solution of 83 mg (0.132 mmol) squamocin **1** in 1.5 mL EtOAc were added 251 mg (0.66 mmol) $N_\alpha$-BOC-$N_\omega$-*Z*-lysine and catalytic 4-DMAP. The mixture was cooled to 0 °C and a solution of 136 mg (0.660 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction media was brought back to room

temperature and stirred for 15 h, then evaporated under reduced pressure. The residue was retaken in a toluene/cyclohexane 50:50 v/v mixture (10 mL) and filtered over Whatman GF/A. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 50:50 v/v), furnishing 189 mg (83%) triester **4p** as a colorless resin. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.87 (t, 3H, H-34, $J$ = 6.8 Hz), 1.26 (m, 2H, H-26), 1.37 (m, 6H, $H_\delta$), 1.39 (d, 3H, H-37, $J$ = 6.8 Hz), 1.42 (s, 27H, $^t$Bu–O), 1.52 (m, 7H, H-4, H-14, $H_\beta$), 1.54 (m, 4H, H-27, H-29), 1.59 (m, 3H, $H_\beta$), 1.64 (m, 4H, H-18, H-21), 1.79 (m, 6H, $H_\gamma$), 1.88 (m, 4H, H-17, H-22), 2.25 (t, 2H, H-3, $J$ = 7 Hz), 3.17 (m, 6H, $H_\varepsilon$), 3.82 (m, 2H, H-19, H-20), 3.95 (m, 2H, H-16, H-23), 4.22 (m, 3H, $H_\alpha$), 4.86 (m, 2H, H-24, H-28), 4.92 (m, 1H, H-15), 4.99 (dq, 1H, H-36, $J$ = 1.5 Hz, $J$ = 6.8 Hz), 5.08 (s, 6H, $H_{Bn}$), 5.15 (m, 3H, $NH_{BOC}$), 6.97 (d, 1H, H-35, $J$ = 1.5 Hz), 7.28–7.36 (*m*, 16H, H-arom.). ESIMS *m*/*z* 1733 $[M+Na]^+$, 878 $[M+2Na]^{2+}$. $[\alpha]_D$ +6 (*c* 1, $CHCl_3$).

**4.2.6. 15,24,28-Tri-*O*-($N_\omega$-*Z*-lysyl)-squamocin tri-(ammonium fluoride) 4a.** A solution of 70 mg (40.9 μmol) triester **4p** in 0.6 mL anisole was cooled to 0 °C and 0.7 mL of a 65% HF solution in pyridine was added dropwise. The reaction medium was stirred at that temperature for 5 h then treated with MeOH (10 mL) and evaporated to dryness. The residue was suspended in water (8 mL), and the precipitate filtered, washed by cyclohexane and air-dried. Thirty four milligrams (57%) of triester **4a** were obtained as an amorphous white powder. $^1$H NMR ($(CD_3)_2CO$), 400 MHz $\delta$ 0.88 (t, 3H, H-34), 1.36 (d, 3H, H-37, $J$ = 7 Hz), 1.55 (m, 2H, H-4), 1.73 (m, 4H, H-18, H-21), 1.91 (m, 4H, H-17, H-22), 2.21 (t, 2H, H-3, $J$ = 7 Hz), 3.12 (m, 6H, $H_\varepsilon$), 3.84 (m, 2H, H-19, H-20), 3.96 (m, 2H, H-16, H-23), 4.07 (m, 3H, $H_\alpha$), 4.83 (m, 2H, H-24, H-28), 4.92 (m, 1H, H-15), 5.03 (m, 7H, H-36, $H_{Bn}$), 7.24–7.41 (m, 16H, H-35, H-arom.). ESIMS *m*/*z* 1431 $[M+Na]^+$, 728 $[M+2Na]^{2+}$. HRESIMS *m*/*z* 1408.8754 (calcd for $C_{79}H_{120}N_6O_{16}$: 1408.8761).

**4.2.7. 15,24,28-Tri-*O*-($N_\alpha$-BOC-lysyl)-squamocin 4b.** To a solution of 74 mg (43.3 μmol) triester **4p** in 0.5 mL absolute EtOH were added 222 mg (3 mass equiv) Pd–C (10%). The mixture was Ar purged; then 150 μL (1.60 mmol) 1,4-cyclohexadiene was added dropwise. The reaction medium was heated to 40 °C and stirred for 2 h at that temperature, then filtered over Whatman GF/A. The filtrate was evaporated under reduced pressure, the residue retaken in 3 mL MeOH and filtered over Celite®-545, furnishing 46 mg (86%) triester **4b** as a white resin. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.87 (t, 3H, H-34, $J$ = 6.8 Hz), 1.35 (d, 3H, H-37, $J$ = 6.8 Hz), 1.43 (m, 6H, $H_\delta$), 1.68 (m, 4H, H-18, H-21), 1.71 (m, 6H, $H_\beta$), 1.92 (m, 10H, $H_\gamma$, H-17, H-22), 2.23 (t, 2H, H-3, $J$ = 7.1 Hz), 2.68 (m, 6H, $H_\varepsilon$), 3.81 (m, 2H, H-19, H-20), 3.94 (m, 2H, H-16, H-23), 4.20 (m, 3H, $H_\alpha$), 4.84 (m, 2H, H-24, H-28), 4.88 (m, 1H, H-15), 4.94 (dq, 1H, H-36, $J$ = 1.5 Hz, $J$ = 6.8 Hz), 5.19 (m, 3H, $NH_{BOC}$), 6.95 (d, 1H, H-35, $J$ = 1.5 Hz). ESIMS *m*/*z* 1309 $[M+H]^+$. $[\alpha]_D$ −8 (*c* 1, EtOAc).

**4.2.8. 15,24,28-Tri-*O*-($N_\alpha$-BOC-prolyl)-squamocin 5p.** To a solution of 86 mg (0.138 mmol) squamocin **1** in 2 mL EtOAc were added 149 mg (0.693 mmol) $N_\alpha$-BOC proline and catalytic 4-DMAP. The mixture was cooled to 0 °C and a solution of 142 mg (0.690 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction media was brought back to room temperature and stirred for 20 h, then evaporated under reduced pressure. The residue was retaken in a toluene/cyclohexane 70:30 v/v mixture (10 mL) and filtered over cotton. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 50:50 v/v), furnishing 120 mg (71%) triester **5p** as a colorless resin. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.86 (t, 3H, H-34, $J$ = 6.8 Hz), 1.30 (m, 2H, H-26), 1.43 (s, 27H, $^t$Bu–O), 1.40 (d, 3H, H-37, $J$ = 6.8 Hz), 1.55 (m, 10H, H-4, H-14, H-25, H-27, H-29), 1.69 (m, 4H, H-18, H-21), 1.89 (m, 6H, $H_\gamma$), 1.93 (m, 4H, H-17, H-22), 1.94 (m, 3H, $H_\beta$), 2.19 (m, 3H, $H_\beta$), 2.26 (t, 2H, H-3, $J$ = 7.3 Hz), 3.48 (m, 3H, $H_\delta$), 3.52 (m, 3H, $H_\delta$), 3.85 (m, 2H, H-19, H-20), 3.98 (m, 2H, H-16, H-23), 4.29 (m, 3H, $H_\alpha$), 4.85 (m, 1H, H-28), 4.87 (m, 2H, H-15, H-24), 4.99 (dq, 1H, H-36, $J$ = 1.5 Hz, $J$ = 6.8 Hz), 6.98 (d, 1H, H-35, $J$ = 1.5 Hz). ESIMS *m*/*z* 1238 $[M+Na]^+$. $[\alpha]_D$ −17 (*c* 1, $CHCl_3$).

**4.2.9. 15,24,28-Tri-*O*-(prolyl)-squamocin tri-(ammonium trifluororacetate) 5.** A solution of 77 mg (63.4 μmol) triester **5p** in 0.4 mL $CH_2Cl_2$ was cooled to 0 °C and 0.8 mL TFA was added dropwise. The reaction medium was stirred at that temperature for 2 h then evaporated under reduced pressure. The crude product was chromatographed over a column of Sephadex® LH-20 ($CH_2Cl_2$ 100%), furnishing 44 mg (55%) triester **5** as a white resin. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.86 (t, 3H, H-34, $J$ = 6.9 Hz), 1.39 (d, 3H, H-37, $J$ = 6.8 Hz), 1.53 (m, 10H, H-4, H-14, H-25, H-27, H-29), 1.82 (m, 4H, H-18, H-21), 1.96 (m, 6H, $H_\gamma$), 2.08 (m, 4H, H-17, H-22), 2.10 (m, 3H, $H_\beta$), 2.41 (m, 3H, $H_\beta$), 2.24 (*t*, 2H, H-3, $J$ = 7.7 Hz), 3.45 (m, 3H, $H_\delta$), 3.74 (*m*, 2H, H-19, H-20), 3.95 (m, 2H, H-16, H-23), 4.51 (m, 3H, $H_\alpha$), 4.89 (m, 3H, H-15, H-24, H-28), 4.98 (dq, 1H, H-36, $J$ = 1.3 Hz, $J$ = 6.8 Hz), 6.96 (d, 1H, H-35, $J$ = 1.3 Hz). ESIMS *m*/*z* 916 $[M+H]^+$. HRESIMS *m*/*z* 913.6396 (calcd for $C_{52}H_{87}N_3O_{10}$: 913.6391).

**4.2.10. 15,24,28-Tri-*O*-($N_\alpha$-BOC-*O*-Bn-seryl)-squamocin 6p.** To a solution of 95 mg (0.153 mmol) squamocin **1** in 1 mL EtOAc were added 226 mg (0.765 mmol) $N_\alpha$-BOC-*O*-Bn-serine and catalytic 4-DMAP. The mixture was cooled to 0 °C and a solution of 158 mg (0.767 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction media was brought back to room temperature and stirred for 6 h, then evaporated under reduced pressure. The residue was retaken in cyclohexane (3 × 3 mL) and filtered over Whatman GF/A. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 35:65 v/v), furnishing 180 mg (81%) triester **6p** as a colorless resin. $^1$H NMR ($CDCl_3$, 400 MHz) $\delta$ 0.85 (t, 3H, H-34, $J$ = 6.8 Hz), 1.22 (m, 2H, H-26), 1.40 (d, 3H, H-37, $J$ = 6.8 Hz), 1.43 (s, 27H, $^t$Bu–O), 1.52 (m, 2H,

H-25), 1.54 (m, 6H, H-4, H-27, H-29), 1.58 (m, 2H, H-14), 1.63 (m, 4H, H-18, H-21), 1.88 (m, 4H, H-17, H-22), 2.25 (t, 2H, H-3, $J$ = 7.1 Hz), 3.68 (m, 3H, $H_\beta$), 3.77 (m, 2H, H-19, H-20), 3.85 (m, 4H, $H_\beta$, H-16/H-23), 3.97 (m, 1H, H-16/H-23), 4.42 (m, 3H, $H_\alpha$), 4.52 (s, 6H, $H_{Bn}$), 4.90 (m, 3H, H-15, H-24, H-28), 4.98 (dq, 1H, H-36, $J$ = 1.5 Hz, $J$ = 6.8 Hz), 5.41 (m, 3H, $NH_{BOC}$), 6.98 (*d*, 1H, H-35, $J$ = 1.5 Hz), 7.25–7.34 (m, 15H, H-arom.). ESIMS *m/z* 1478 $[M+Na]^+$, 750 $[M+2Na]^{2+}$. $[\alpha]_D$ +27 (*c* 1, $CHCl_3$).

**4.2.11. 15,24,28-Tri-*O*-($N_\alpha$-BOC-seryl)-squamocin 6.** To a solution of 87 mg (59.8 μmol) triester **6p** in 1 mL absolute EtOH were added 261 mg (3 mass equiv) Pd–C (10%). The mixture was Ar purged; then 175 μL (1.86 mmol) 1,4-cyclohexadiene was added dropwise. The reaction medium was heated to 45 °C and stirred for 5 h at that temperature, then filtered over Whatman GF/A. The filtrate was evaporated under reduced pressure and the residue chromatographed over a small column of silica gel (EtOAc/cyclohexane 70:30 v/v), furnishing 37 mg (52%) triester **6** as a white resin. $^1H$ NMR ($CD_2Cl_2$, 400 MHz) $\delta$ 0.86 (t, 3H, H-34, $J$ = 6.8 Hz), 1.27 (m, 2H, H-26), 1.37 (d, 3H, H-37, $J$ = 6.8 Hz), 1.44 (s, 27H, $^t$Bu–O), 1.51 (m, 2H, H-25), 1.49 (m, 2H, H-14), 1.54 (m, 6H, H-4, H-27, H-29), 1.54 (m, 4H, H-18, H-21), 1.78 (m, 4H, H-17, H-22), 2.22 (t, 2H, H-3, $J$ = 7.3 Hz), 3.68 (m, 2H, H-19, H-20), 3.75 (m, 3H, $H_\beta$), 3.93 (m, 3H, $H_\beta$), 3.96 (m, 1H, H-23), 3.99 (m, 1H, H-16), 4.12 (m, 1H, $H_\alpha$), 4.31 (*m*, 2H, $H_\alpha$), 4.86 (m, 1H, H-24), 4.94 (m, 1H, H-28), 4.97 (dq, 1H, H-36, $J$ = 1.5 Hz, $J$ = 6.8 Hz), 5.09 (m, 1H, H-15), 5.47–5.69 (m, 3H, $NH_{BOC}$), 6.99 (d, 1H, H-35, $J$ = 1.5 Hz). ESIMS *m/z* 1202 $[M+NH_4]^+$. $[\alpha]_D$ −6 (*c* 0.5, EtOAc).

**4.2.12. 15,24,28-Tri-*O*-($N_\alpha$-BOC-*O*-Bn-tyrosyl)-squamocin 7p.** To a solution of 101 mg (0.162 mmol) squamocin **1** in 1.5 mL EtOAc were added 301 mg (0.812 mmol) $N_\alpha$-BOC-*O*-Bn-tyrosine and catalytic 4-DMAP. The mixture was cooled to 0 °C and a solution of 167 mg (0.812 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction media was brought back to room temperature and stirred for 15 h, then evaporated under reduced pressure. The residue was retaken in a toluene/cyclohexane 20:80 v/v (3 × 5 mL) and filtered over Whatman GF/A. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 30:70 v/v), furnishing 208 mg (76%) triester **7p** as a colorless resin. $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 0.87 (t, 3H, H-34, $J$ = 6.8 Hz), 1.26 (m, 2H, H-26), 1.39 (s, 27H, $^t$Bu–O), 1.42 (d, 3H, H-37, $J$ = 6.8 Hz), 1.50 (m, 4H, H-27, H-29), 1.54 (m, 6H, H-4, H-14, H-25), 1.72 (m, 4H, H-18, H-21), 1.89 (m, 4H, H-17, H-22), 2.25 (t, 2H, H-3, $J$ = 7.1 Hz), 2.92 (m, 3H, $H_\beta$), 3.09 (m, 3H, $H_\beta$), 3.84 (m, 2H, H-19, H-20), 3.99 (m, 2H, H-16, H-23), 4.49 (m, 3H, $H_\alpha$), 4.85 (m, 2H, H-24, H-28), 4.96 (dq, 1H, H-36), 4.97 (m, 3H, $NH_{BOC}$), 5.01 (m, 1H, H-15), 5.02 (s, 6H, $H_{Bn}$), 6.88 (m, 6H, $H_b$), 6.96 (d, 1H, H-35), 7.08 (m, 6H, $H_a$), 7.25–7.43 (m, 15H, H-arom.). ESIMS *m/z* 1706 $[M+Na]^+$. $[\alpha]_D$ +22 (*c* 1, $CHCl_3$).

**4.2.13. 15,24,28-Tri-*O*-($N_\alpha$-BOC-tyrosyl)-squamocin 7.** To a solution of 66 mg (39.2 μmol) triester **7p** in 0.6 mL absolute EtOH were added 198 mg (3 mass equiv) Pd–C (10%). The mixture was Ar purged; then 200 μL (2.14 mmol) 1,4-cyclohexadiene was added dropwise. The reaction medium was heated to 65 °C and stirred for 8 h at that temperature, then filtered over Whatman GF/A. The filtrate was evaporated under reduced pressure and the residue chromatographed over a small column of silica gel (EtOAc/cyclohexane 55:45 v/v), furnishing 33 mg (60%) triester **7** as a white resin. $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 0.84 (t, 3H, H-34, $J$ = 6.8 Hz), 1.41 (s, 27H, $^t$Bu–O), 1.41 (d, 3H, H-37, $J$ = 6.8 Hz), 1.54 (m, 4H, H-4, H-14), 1.69 (m, 4H, H-18, H-21), 1.90 (m, 4H, H-17, H-22), 2.22 (t, 2H, H-3, $J$ = 7 Hz), 2.98 (m, 6H, $H_\beta$), 3.83 (m, 3H, H-19, H-20, H-16/H-23), 3.97 (m, 1H, H-16/H-23), 4.47 (m, 3H, $H_\alpha$), 4.71 (m, 1H, H-24/H-28), 4.83 (m, 1H, H-24/H-28), 4.95 (m, 3H, $NH_{BOC}$), 4.96 (dq, 1H, H-36, $J$ = 1.3 Hz, $J$ = 6.8 Hz), 5.14 (m, 1H, H-15), 6.72 (m, 6H, $H_b$), 6.97 (m, 7H, $H_a$, H-35). ESIMS *m/z* 1430 $[M+NH_4]^+$. HRESIMS *m/z* 1411.8289 (calcd for $C_{79}H_{117}N_3O_{19}$: 1411.8281). $[\alpha]_D$ +6 (*c* 1, EtOAc).

**4.2.14. 15,24,28-Tri-*O*-($N_\alpha$-BOC-*O*-Bn glutamyl)-squamocin 8p.** To a solution of 112 mg (0.180 mmol) squamocin **1** in 2 mL EtOAc were added 303 mg (0.9 mmol) $N_\alpha$-BOC-*O*-Bn-glutamate and catalytic 4-DMAP. The mixture was cooled to 0 °C and a solution of 185 mg (0.90 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction media was brought back to room temperature and stirred for 24 h, then evaporated under reduced pressure. The residue was retaken in a toluene (3 × 5 mL) and filtered over Whatman GF/A. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 35:65 v/v), furnishing 193 mg (68%) triester **8p** as a colorless resin. $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 0.86 (t, 3H, H-34, $J$ = 6.8 Hz), 1.27 (m, 2H, H-26), 1.40 (d, 3H, H-37, $J$ = 6.8 Hz), 1.43 (s, 27H, $^t$Bu–O), 1.53 (m, 8H, H-4, H-14, H-27, H-29), 1.68 (m, 4H, H-18, H-21), 1.91 (m, 4H, H-17, H-22), 1.93 (m, 3H, $H_\beta$), 2.20 (m, 3H, $H_\beta$), 2.25 (t, 2H, H-3, $J$ = 7.3 Hz), 2.46 (m, 6H, $H_\gamma$), 3.79 (m, 2H, H-19, H-20), 3.93 (m, 2H, H-16, H-23), 4.32 (m, 3H, $H_\alpha$), 4.86 (m, 2H, H-24, H-28), 4.97 (m, 1H, H-15), 4.99 (dq, 1H, H-36, $J$ = 1.5 Hz, $J$ = 6.8 Hz), 5.11 (s, 6H, $H_{Bn}$), 5.15 (m, 3H, $NH_{BOC}$), 6.97 (d, 1H, H-35, $J$ = 1.5 Hz), 7.30–7.35 (m, 15H, H-arom.). ESIMS *m/z* 1604 $[M+Na]^+$, 813 $[M+2Na]^{2+}$. $[\alpha]_D$ +5 (*c* 1, $CHCl_3$).

**4.2.15. 15,24,28-Tri-*O*-($N_\alpha$-BOC-glutamyl)-squamocin 8.** To a solution of 90 mg (56.9 μmol) triester **8p** in 1 mL absolute EtOH were added 270 mg (3 mass equiv) Pd–C (10%). The mixture was Ar purged; then 300 μL (3.19 mmol) 1,4-cyclohexadiene was added dropwise. The reaction medium was heated to 40 °C and stirred for 3 h at that temperature, then filtered over Whatman GF/A. The filtrate was evaporated under reduced pressure and the residue chromatographed over a column of alumina (toluene/MeOH/AcOH 90:7:3 v/v/v), furnishing 24 mg (32%) triester **8** as a colorless resin. $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 0.84 (t, 3H, H-34, $J$ = 6,8 Hz), 1.35

(d, 3H, H-37, $J = 6.8$ Hz), 1.92 (m, $H_\beta$, 14H, H-17, H-18, H-21, H-22), 2.25 (t, 2H, H-3, $J = 7$ Hz), 2.42 (m, 6H, $H_\gamma$), 3.83 (m, 2H, H-19, H-20), 4.02 (m, 2H, H-16, H-23), 4.32 (m, 3H, $H_\alpha$), 4.76 (m, 1H, H-24/H-28), 4.87 (m, 1H, H-24/H-28), 4.93 (m, 1H, H-15), 5.0 (dq, 1H, H-36, $J = 1.3$ Hz, $J = 6.8$ Hz), 5.33 (m, 3H, $NH_{BOC}$), 6.98 (d, 1H, H-35, $J = 1.3$ Hz). ESIMS *m/z* 1333 $[M+Na]^+$.

**4.2.16. 15,24,28-Tri-*O*-($N_\alpha$-BOC $N_{im}$-Ts-histidyl)-squamocin 9p.** To a solution of 134 mg (0.215 mmol) squamocin **1** in 2 mL EtOAc were added 441 mg (0.9 mmol) $N_\alpha$-BOC-$N_{im}$-Ts-histidine and catalytic 4-DMAP. The mixture was cooled to 0 °C and a solution of 222 mg (1.077 mmol) DCC in 1 mL EtOAc was added dropwise. The reaction media was brought back to room temperature and stirred for 30 h, then evaporated under reduced pressure. The residue was retaken in THF ($3 \times 7$ mL) and filtered over Whatman GF/A. The filtrate was evaporated in vacuo and the crude product chromatographed over a column of silica gel (EtOAc/cyclohexane 60:40 v/v), furnishing 143 mg (37%) triester **9p** as a white resin. $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 0.85 (t, 3H, H-34, $J = 6.9$ Hz), 1.22 (m, 2H, H-26), 1.38 (s, 27H, $^t$Bu–O), 1.40 (d, 3H, H-37, $J = 6.8$ Hz), 1.49 (m, 2H, H-14), 1.51 (m, 4H, H-27, H-29), 1.53 (m, 4H, H-4, H-25), 1.67 (m, 4H, H-18, H-21), 1.89 (m, 4H, H-17, H-22), 2.25 (t, 2H, H-3, $J = 7.4$ Hz), 2.42 (s, 9H, $CH_{3Ts}$), 2.98 (m, 6H, $H_\beta$), 3.83 (m, 2H, H-19, H-20), 3.93 (m, 2H, H-16, H-23), 4.51 (m, 3H, $H_\alpha$), 4.81 (m, 2H, H-24, H-28), 4.84 (m, 1H, H-15), 4.97 (dq, 1H, H-36, $J = 1.6$ Hz, $J = 6.8$ Hz), 5.64 (m, 3H, $NH_{BOC}$), 6.97 (d, 1H, H-35, $J = 1.6$ Hz), 7.09 (s, 1H, H-4′), 7.10 (s, 1H, H-4′), 7.15 (s, 1H, H-4′), 7.33–7.79 (m, 12H, $H_{Ts}$), 7.88 (s, 1H, H-2′), 7.89 (s, 2H, H-2′). ESIMS *m/z* 921 $[M+2Na]^{2+}$. $[\alpha]_D$ +10 (*c* 1, $CHCl_3$).

**4.2.17. 15,24,28-Tri-*O*-($N_\alpha$-BOC-histidyl)-squamocin 9.** To a solution of 180 mg (0.10 mmol) triester **9p** in 1.5 mL anhydrous THF were added 162 mg (1.2 mmol) HOBT, and the mixture was stirred at room temperature for 5 h. The reaction medium was filtered over Whatman GF/A, and the filtrate evaporated under reduced pressure. The residue was filtered over alumina (EtOAc/MeOH 90:10 v/v) and the crude product chromatographed in the same conditions, furnishing 56 mg (42%) **9** as a colorless resin. $^1H$ NMR ($CDCl_3$, 200 MHz) $\delta$ 0.84 (t, 3H, H-34), 1.35 (s, 27H, $^t$Bu–O), 1.39 (d, 3H, H-37, $J = 6.8$ Hz), 1.91 (m, 4H, H-17, H-22), 2.22 (t, 2H, H-3, $J = 7$ Hz), 3.15 (m, 6H, $H_\beta$), 3.83 (m, 2H, H-19, H-20), 3.96 (m, 2H, H-16, H-23), 4.43 (m, 3H, $H_\alpha$), 4.79 (m, 3H, H-15, H-24, H-28), 4.96 (dq, 1H, H-36, $J = 1.3$ Hz, $J = 6.8$ Hz), 5.50 (m, 3H, $NH_{BOC}$), 6.89 (s, 2H, H-4′), 6.96 (d, 1H, H-35, $J = 1.3$ Hz), 7.05 (s, 1H, H-4′), 8.27 (s, 3H, H-2′). ESIMS 1335 $[M+H]^+$. HRESIMS *m/z* 1333.8143 (calcd for $C_{70}H_{111}N_9O_{16}$: 1333.8149).

### 4.3. Bioassays

**4.3.1. Mitochondrial complex I inhibition.** The enzymatic inhibitory activities of the squamocin **1** triesters were evaluated as described elsewhere.[14,31]

**4.3.2. Cytotoxicities.** Cytotoxicities were colorimetrically evaluated through a 96 wells plate assay after 72 h cell exposure to the acetogenins. KB 3-1 cells were cultivated in Dullbecco's MEM 'glutamax' (1 g/L glucose) enriched with 10% FCS, in a moist atmosphere containing 5% $CO_2$. Each well of the test plate was inseminated at $t_0$ with 100 μL of a $1.2 \times 10^5$ cell/mL cellular suspension. Homogeneous dilutions were obtained from a freshly prepared $10^{-3}$ M solution of the acetogenin in DMSO, a 100 μL aliquote of this solution being diluted in 5.90 mL of the culture media. This obtained $2 \times 10^{-5}$ M solution was further and extensively diluted with 2% DMSO-precontaining culture media. After 24 h cell growth, 100 μL of the dilutions were administrated in triplicate into the wells. Final homogenization was ensured by a 3 min treatment on a microtitration plate. After 72 h contact, the test plates were emptied and directly treated with 200 μL of a 2.5 g/L methylene blue solution in MeOH/water 50:50 v/v after the method of Fleury et al.[32] After 40 min contact, the plates were emptied and the wells quickly rinsed under a very mild flow of cold tap water (three times). The plates were air-dried (50 °C) and each well treated with 100 μL of 0.1 N HCl. Absorbances were recorded at 620 nm after 30 min incubation at 37 °C and homogenization on a microtitration plate, under a blank of 100 μL of 0.1 N HCl. $IC_{50}$ were expressed as the extrapolated concentrations allowing 50% cell survival in comparison with untreated controls.

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