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Synthesis, structure–activity relationships, and mechanism of action of anti-HIV-1 lamellarin α 20-sulfate analogues

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ABSTRACT

Lamellarin α and six different types of lamellarin α 20-sulfate analogues were synthesized and their structure–activity relationships were investigated using a single round HIV-1 vector infection assay. All lamellarin sulfates having pentacyclic lamellarin core exhibited anti-HIV-1 activity at a 10 μM concentration range regardless of the number and position of the sulfate group. On the other hand, non-sulfated lamellarin α and ring-opened lamellarin sulfate analogues did not affect HIV-1 vector infection in similar concentrations. The lamellarin sulfates utilized in this study did not exhibit unfavorable cytotoxic effect under the concentrations tested (IC_{50} > 100 μM). Confocal laser scanning microscopic analysis indicated that hydrophilic lamellarin sulfates were hardly incorporated in the cell. HIV-1 Env-mediated cell–cell fusion was suppressed by lamellarin sulfates. These results suggested that lamellarin sulfates have a novel anti-HIV-1 activity besides the previously reported integrase activity inhibition, possibly at a viral entry step of HIV-1 replication.

1. Introduction

Lamellarins are polyaromatic marine alkaloids that possess a unique 14-phenyl-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one framework.[1] Up to now, approximately 40 lamellarins have been isolated from mollusks, tunicates, and sponges.[2] These differ in the number and position of the OH and OMe groups on the common structural core. Lamellarins exhibit a wide range of useful biological activities; most notable is potent cytotoxic activity against cancer cell lines, including the MDR phenotype.[3] Extensive studies on structure–activity relationship have revealed the structural requirements for the cytotoxicity.[4] Multiple cellular targets, such as DNA topoisomerase I,[5] cancer-relevant protein kinases,[6] and mitochondria,[7] have been discovered. Some lamellarin derivatives are currently under preclinical development as anti-cancer agents.[8]

Anti-HIV-1 activity is another important biological effect of lamellarins. Faulkner and coworkers screened diverse natural marine products for compounds active against HIV-1 integrase in vitro and found that some lamellarins showed selective inhibition for this enzyme.[9] Within the series of lamellarins tested, lamellarin α 20-sulfate displayed the most favorable therapeutic index. This compound inhibited integrase terminal cleavage activity with an IC_{50} of 16 μM, strand transfer activity with an IC_{50} of 22 μM, and growth of the HIV-1 virus in cell culture with an IC_{50} of 8 μM. The MTT assay of lamellarin α 20-sulfate toward HeLa cells displayed the least toxicity of 274 μM.

Despite the promising feature of lamellarin α 20-sulfate as a new lead anti-HIV-1 agent, the structure–activity relationships of lamellarin sulfates have scarcely been investigated.[10] This is presumably due to the lack of a methodology for the synthesis of regioselectively sulfated lamellarins. Recently, however, we have developed a method to overcome this problem and achieved the first total synthesis of lamellarin α 20-sulfate[11] and related compounds.[12] In the present study, we have synthesized non-sulfated lamellarin α (**1**) and six different types of sulfated lamellarins **2**–**7** and investigated the structure–activity relationships using single-round HIV-1 vector infection assay (Fig. 1). A plausible mechanism of action of the lamellarin sulfates in vivo is also proposed.

2. Results and discussion

2.1. Synthesis

The synthesis of lamellarin α (**1**), ring-opened lamellarin sulfate (**2**), lamellarin U 20-sulfate (**3**), lamellarin α 20-sulfate (**4**), and

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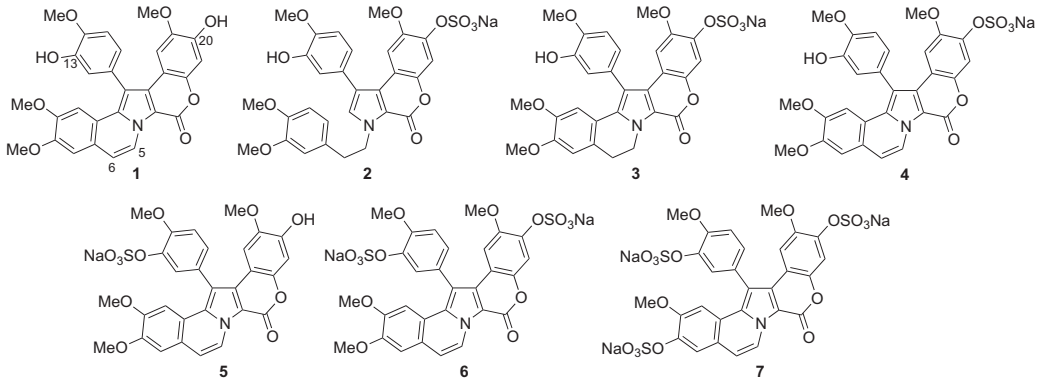
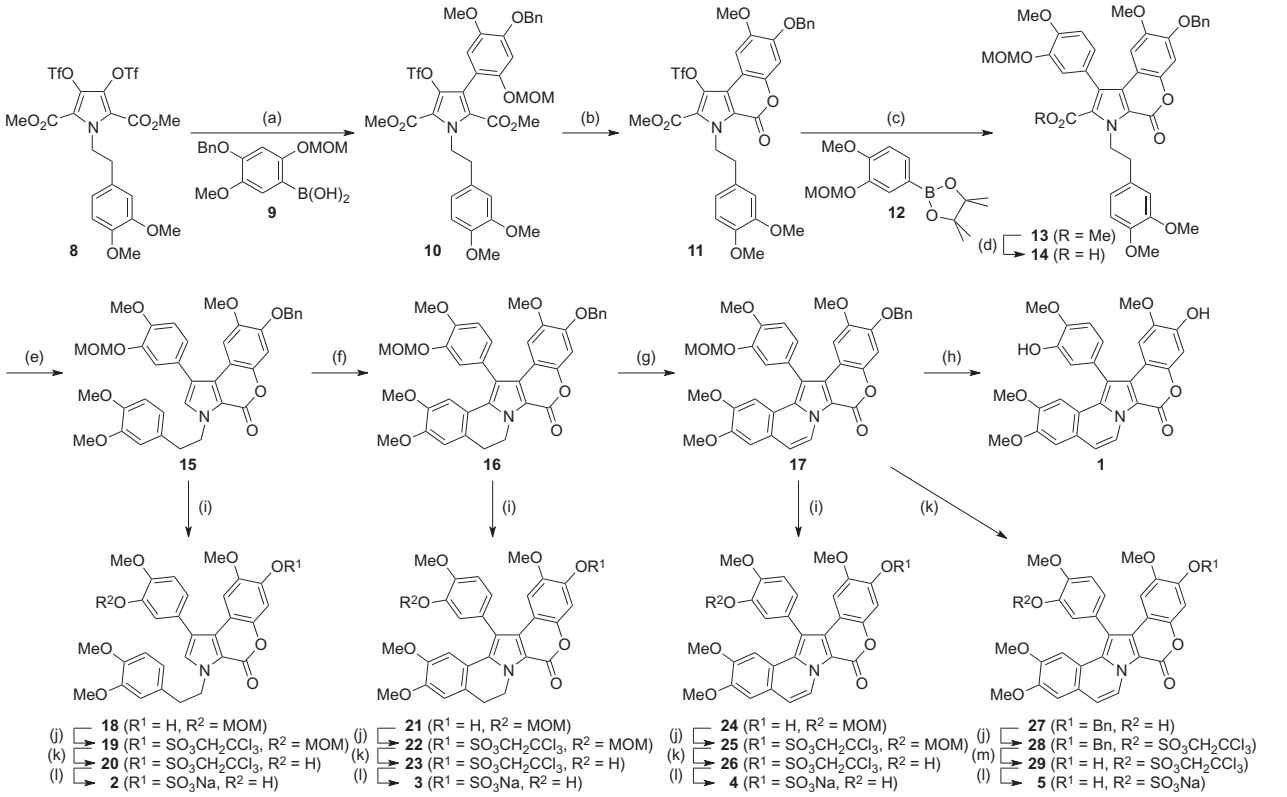


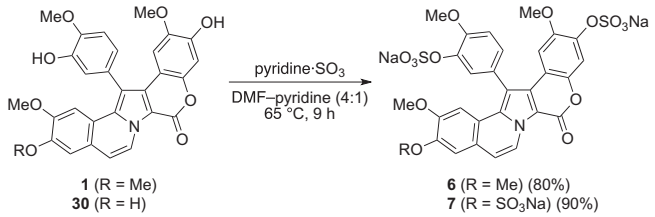
Figure 1. Lamellarin α (**1**) and lamellarin sulfates **2**–**7** utilized in this study.

lamellarin α 13-sulfate (**5**) is show in Scheme 1. Palladium-catalyzed Suzuki–Miyaura coupling of the known 3,4-dihydroxypyrrole bistriflate **8**[13] with 1.2 equiv of arylboronic acid **9** gave the mono-arylated pyrrole **10** in 77% yield. This compound was converted to **11** in 96% yield by deprotection of the methoxymethyl (MOM) group followed by acid-catalyzed lactonization. The second Suzuki–Miyaura coupling of **11** with 1.3 equiv of pinacol borate **12** gave 3,4-differentially arylated pyrrole **13** in 94% yield. Alkaline hydrolysis of **13** followed by pyridinium *p*-toluenesulfonate (PPTS)-catalyzed relactonization afforded the acid **14** in 73% yield. Decarboxylation of **14** in hot quinoline in the presence of copper(I) oxide provided **15** in nearly quantitative yield. Intramolecular oxidative biaryl coupling[14] of **15** in the presence of phenyliodine bis(trifluoroacetate) (PIFA)-boron trifluoride diethyl etherate gave the lamellarin framework **16** in 54% yield. Treatment of this compound with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing dichloromethane afforded 5,6-unsaturated lamellarin **17** in 87% yield. Simultaneous deprotection of both benzyl and MOM groups with 6 equiv of boron trichloride produced lamellarin α (**1**) in 98% yield.

Regioselective installation of the sulfate group onto the heterocyclic scaffold of **15**, **16**, and **17** was effected by selective removal



Scheme 1. Synthesis of lamellarin α (**1**) and sulfated lamellarins **2**–**5**. Reagents and conditions: (a) **9**, $Pd(PPh_3)_4$, Na_2CO_3, water, THF, reflux, 3 h (77%); (b) (1) concd HCl, MeOH, reflux, 3 h, (2) *p*-TsOH, CH_2Cl_2, reflux, 3.5 h (96%); (c) **12**, $Pd(PPh_3)_4$, Na_2CO_3, water, THF, reflux, 3 h (94%); (d) (1) 40% aqueous KOH, EtOH, reflux, 2 h, (2) PPTS, CH_2Cl_2, reflux, 8 h (73%); (e) Cu_2O, quinoline, 220 °C, 10 min (99%); (f) PIFA, $BF_3{\cdot}OEt_2$, CH_2Cl_2, −40 °C, 1.5 h (54%); (g) DDQ, CH_2Cl_2, reflux, 30 h (87%); (h) BCl_3, CH_2Cl_2, −78 °C, 0.5 h then 0 °C, 3 h (98%); (i) H_2, 10% Pd-C, EtOAc (**18**: 99%, **21**: 98%, **24**: 91%); (j) $CCl_3CH_2OSO_2Cl$, Et_3N, DMAP, CH_2Cl_2, (**19**: 90%, **22**: 99%, **25**: 99%, **28**: 98%); (k) concd HCl, MeOH–CH_2Cl_2 (1:2) (**20**: 94%, **23**: 92%, **26**: 98%, **27**: 97%); (l) (1) Zn powder, HCO_2NH_4, THF–MeOH (1:1), (2) Amberlite IRC-50 (Na^+ form), (3) Sephadex LH-20 (**2**: 90%, **3**: 71%, **4**: 99%, **5**: 55%); (m) BCl_3, CH_2Cl_2, −78 °C, 0.5 h then 0 °C, 1 h (98%).



1 (R = Me)
30 (R = H)

6 (R = Me) (80%)
7 (R = SO_3Na) (90%)

Scheme 2. Synthesis of lamellarin sulfates **6** and **7**.

Table 1
Anti-HIV-1 activity and cytotoxicity of lamellarins **1**–**7**

Entry	Compound	IC_{50} (μM)	
		Anti-HIV-1 activity	Cytotoxicity
1	**1**	>100	–
2	**2**	>100	–
3	**3**	15.3	>100
4	**4**	13.0	>100
5	**5**	14.4	>100
6	**6**	15.1	>100
7	**7**	20.7	>100

of the benzyl or MOM protecting group under different conditions followed by sulfation utilizing the Taylor protocol.[15] For example, the sulfate **2** was synthesized from **15** in four steps as follows. Debenzylation of **15** under hydrogen atmosphere in the presence of 10% palladium on charcoal gave phenol **18** in nearly quantitative yield. This compound was reacted with 2,2,2-trichloroethyl (TCE) chlorosulfate to give the mixed sulfate **19** in 90% yield. Deprotection of MOM under acidic conditions provided another phenol **20** in 94% yield. Final reductive deprotection of 2,2,2-TCE group with zinc powder in the presence of ammonium formate followed by ion exchange over IRC-50 (Na^+ form) and Sephadex purification produced **2** in 90% yield. Lamellarin U 20-sulfate (**3**) and lamellarin α 20-sulfate (**4**) were synthesized using similar transformations starting from **16** and **17**, respectively. Lamellarin α 13-sulfate (**5**), on the other hand, was synthesized by initial deprotection of MOM followed by 2,2,2-trichloroethoxysulfonylation, debenzylation by boron trichloride, and a final reductive removal of TCE moiety.

Lamellarin α 13,20-disulfate (**6**) and lamellarin N 8,13,20-trisulfate (**7**) were synthesized in good yields from lamellarin α (**1**) and lamellarin N (**30**), respectively, by treatment with conventional pyridine–SO_3 complex (Scheme 2).

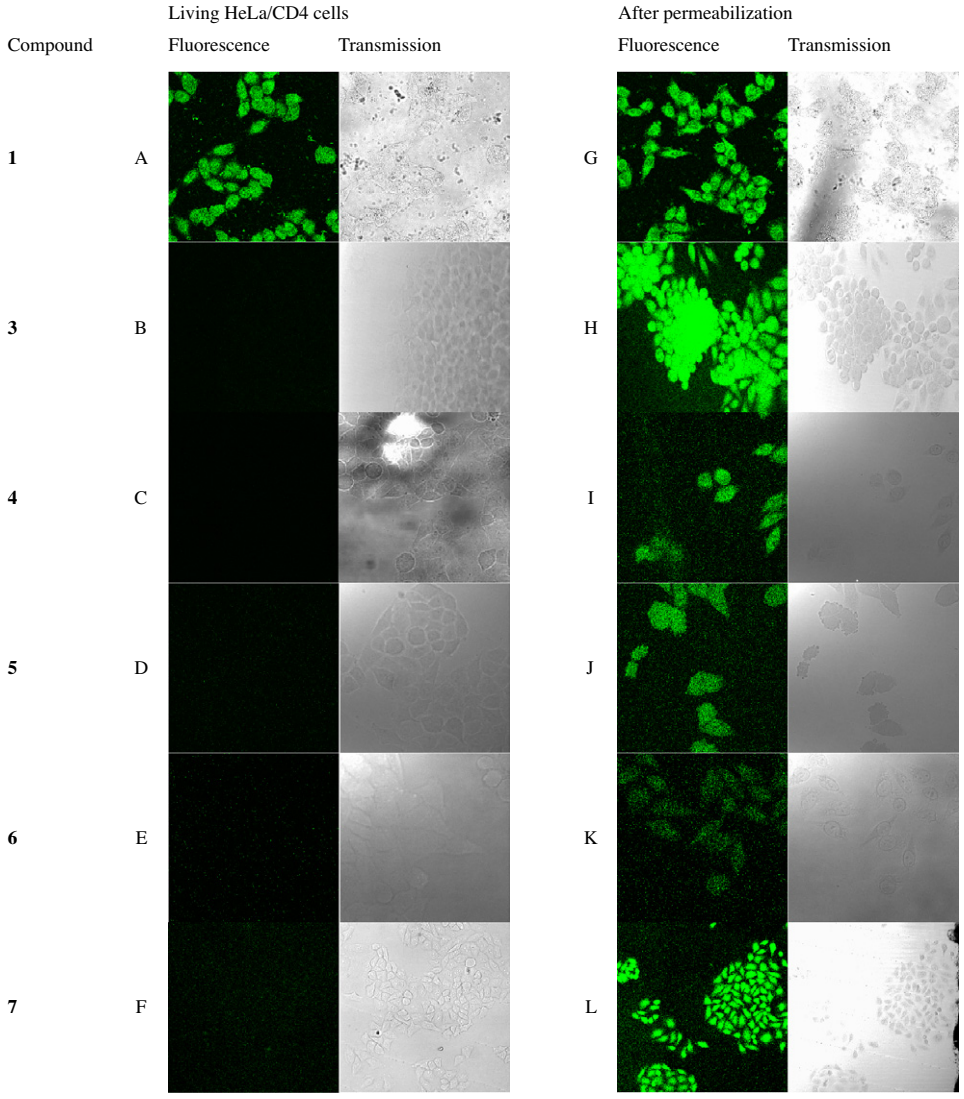


Figure 2. Confocal laser scanning microscopic observation of the cellular uptake of lamellarin α (**1**) and lamellarin sulfates **3**–**7**. Photographs A–F indicate the results using living HeLa/CD4 cells. Photographs G–L indicate the results after permeabilization of the cell membrane by treatment with methanol.

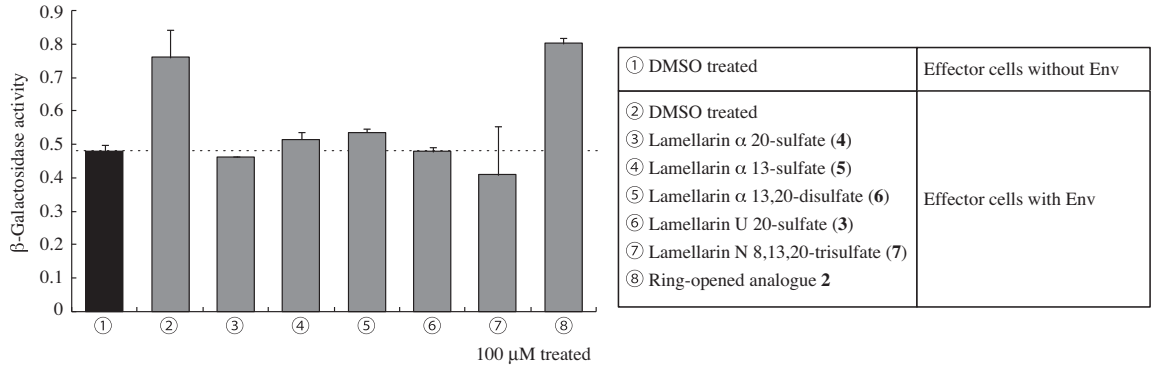


Figure 3. Effect of lamellarin sulfates on HIV-1 Env-mediated cell–cell fusion.

2.2. Anti-HIV-1 evaluation

The anti-HIV-1 activity of lamellarins **1**–**7** was estimated at first by single-round HIV-1 vector infection assays[16] on HeLa/CD4 cells (see Section 4). The IC_{50} values estimated from dose–response curves are summarized in Table 1. Both non-sulfated lamellarin α (**1**) and the ring-opened sulfate (**2**) did not show anti-HIV-1 activity at 100 μM (entries 1 and 2). On the other hand, sulfates **3**–**7** exhibited anti-HIV-1 activities at a 10 μM concentration range (entries 3–7). When the target cells were treated with these compounds at 100 μM, the infected cells were not detected. These results indicated that the presence of both the pentacyclic lamellarin core and the sulfate group is an essential structural requirement for the anti-HIV-1 activity of lamellarins. Other structural elements, such as saturation or unsaturation between C5 and C6 bond (entries 3 and 4), position of the sulfate group in the lamellarin ring (entries 4 and 5), and the number of sulfate groups (entries 4, 5, 6, and 7) are relatively less important.

It has been reported that the parental non-sulfated lamellarins exhibit potent cytotoxicity on a variety of cancer cell lines via multiple mechanism of action.[5–7] Therefore, we performed WST-1 assays to estimate the cytotoxicity of lamellarin sulfates **3**–**7**. HeLa/CD4 cells were incubated with these compounds at 100 μM for 24 h prior to assay. No sulfates tested exhibited unfavorable cytotoxicity, at least under these conditions (Table 1).

The lack of cytotoxicity of sulfates **3**–**7** suggested that these hydrophilic compounds are not incorporated in HeLa/CD4 cells. Recently, Cushman and co-workers demonstrated by LC–MS–MS analysis that resveratrol sulfates, which are structurally similar to lamellarin sulfates, displayed negligible uptake in MCF7 cells.[17] Lamellarins are UV fluorescent (Fig. S2 in Supplementary data). Therefore, we examined cellular uptake of lamellarin sulfates using a confocal laser scanning microscope. HeLa/CD4 cells were treated with lamellarins **1**, **3**–**7** at 100 μM for 24 h. After washing with phosphate-buffered saline (PBS) and fixing in 4% paraformaldehyde, the resulting cells were observed by a confocal laser scanning microscope. The fluorescent signal corresponding to the lamellarin framework was observed in the cells treated with lamellarin α (**1**) (Fig. 2, A).[18] However, no fluorescent signals were detected in the cells treated with lamellarin sulfates **3**–**7** (Fig. 2, B–F). For comparison, similar experiments were carried out after permeabilization of the cell membrane with methanol. In these cases, fluorescent signals were observed in all cells treated with both lamellarin α **1** and lamellarin sulfates **3**–**7** (Fig. 2, G–L). These results clearly indicate that hydrophilic lamellarin sulfates **3**–**7** do not pass through the hydrophobic cell membrane.

Inaccessibility of lamellarin sulfates into HeLa/CD4 cells suggested that these compounds inhibit HIV-1 infection at the virus entry step rather than at the later integration step. HIV-1 gains entry into susceptible cells by fusion of the viral membrane with the cell plasma membrane.[19] The membrane fusion process between HIV-1 and target cells is initiated by the attachment of HIV-1 envelope glycoprotein (Env) to the receptors on the target cell membrane.[20] The HIV-1 Env-expressing cells can fuse with susceptible cells by the membrane fusion activity of the Env protein.[21,22] It is thought that the cell–cell fusion by the Env protein reflects the membrane fusion between virions and target cells.[23,24] Thus, we examined the inhibitory effect of lamellarin sulfates on the HIV-1 Env-mediated cell–cell fusion using a co-culture system of MAGIC 5 target cells and HIV-1 Env-expressing 293T effector cells. The degree of inhibition was estimated by measuring the β-galactosidase activities of the cell lysates. The effects of lamellarin sulfates are summarized as a bar graph in Figure 3. Bar 1 indicates a negative control in which Env-non-expressing effector cells (no ability of cell–cell fusion) were employed. Bar 2 shows a positive control in which Env-expressing effector cells were employed in the absence of inhibitors. On treatment with lamellarin sulfates **3**–**7** at 100 μM (bars 3–7), the β-galactosidase activities of the co-culture systems were reduced to the same as that of the negative control. In contrast, on treatment with ring-opened sulfate **2** at the same concentration (bar 8), the β-galactosidase activity was increased to the level of positive control. These results clearly indicate that lamellarin sulfates **3**–**7** inhibited cell–cell fusion at 100 μM, whereas ring-opened analogue **2** did not. The good correlation between the inhibitory effects on HIV-1 infection and on HIV-1 Env-mediated cell–cell fusion suggested that the mechanism of action of lamellarin sulfates is entry inhibition rather than integrase inhibition.

3. Conclusion

A small library of anti-HIV-1 lamellarin α 20-sulfate analogues was prepared using a synthetic strategy developed in our laboratories. The structure–activity relationship study revealed that the pentacyclic lamellarin core and the sulfate group are essential for anti-HIV-1 activity. Confocal laser microscopic analyses and cell–cell fusion experiments suggested that the anti-HIV-1 activity of lamellarin sulfates are caused by inhibition of the virus entry step rather than the previously indicated integration step.

4. Experimental section

4.1. Synthesis—general

Melting points are uncorrected. IR spectra are reported in terms of frequency of absorption (cm^{-1}). ^1H NMR spectra were recorded

at 400 MHz and are reported relative to Me_4Si (δ 0.0). Data for ^1H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constant (Hz) and integration. ^{13}C NMR spectra were recorded at 100 MHz and are reported relative to Me_4Si (δ 0.0). Data for ^{13}C NMR spectra are reported in terms of chemical shift. High resolution mass spectra were measured by the EI or FAB method. Elemental analysis was performed for C, H, and N. Column chromatography was conducted on silica gel 60 N, 63–210 μm unless otherwise mentioned. Solvents were dried and distilled by standard methods if necessary.

4.2. Synthesis of lamellarin α (1) and sulfated lamellarins 2–7

4.2.1. Dimethyl 3-[4-benzyloxy-5-methoxy-2-(methoxymethoxy)phenyl]-1-[2-(3,4-dimethoxyphenyl)ethyl]-4-(trifluoromethanesulfonyloxy)pyrrole-2,5-dicarboxylate (10)

Under an argon atmosphere, a mixture of **8** (12.9 g, 20.0 mmol), **9** (7.64 g, 24.0 mmol), $Pd(PPh_3)_4$ (462 mg, 0.400 mmol), Na_2CO_3 (14.0 g, 132 mmol), and degassed water (40 mL) in THF (400 mL) was refluxed for 3 h. The mixture was cooled to room temperature and evaporated. The products were extracted with dichloromethane and the extract was washed successively with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (toluene–ethyl acetate = 10:1) to give **10** as pale brown solid (11.8 g, 77%). Mp 113.5–115 °C; IR (KBr) 1728, 1518, 1418, 1239, 1216 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.03 (t, J = 6.9 Hz, 2H), 3.30 (s, 3H), 3.53 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 3.90 (s, 3H), 4.86 (br s, 2H), 4.91–5.01 (m, 2H), 5.18 (s, 2H), 6.70 (d, J = 1.9 Hz, 1H), 6.72 (dd, J = 1.9 and 8.1 Hz, 1H), 6.72 (s, 1H), 6.77 (d, J = 8.1 Hz, 1H), 7.27–7.32 (m, 1H), 7.33–7.38 (m, 2H), 7.43–7.47 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.6, 48.6, 51.7, 51.8, 55.7, 55.8, 56.0, 56.7, 71.2, 96.0, 103.9, 111.4, 112.2, 112.4, 115.1, 117.1, 118.1 (q, J = 320 Hz), 118.4, 121.2, 124.2, 127.7, 127.9, 128.5, 130.3, 136.2, 136.7, 144.6, 147.9, 148.7, 148.9, 149.4, 159.3, 161.1; HRFABMS m/z calcd for $C_{35}H_{36}F_3NO_{13}S$ (M^+) 767.1859, found 767.1855.

4.2.2. Methyl 7-benzyloxy-3-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dihydro-8-methoxy-4-oxo-1-(trifluoromethanesulfonyloxy)-[1]benzopyrano[3,4-*b*]pyrrole-2-carboxylate (11)

To a solution of **10** (11.8 g, 15.3 mmol) in methanol (1.0 L) was added concd HCl (100 mL) at room temperature. After being refluxed for 3 h, the mixture was cooled to room temperature and evaporated. The residue was poured into water (200 mL) and the precipitates thus formed was collected by filtration, washed with water, and dried under reduced pressure. On the other hand, the filtrate was extracted with dichloromethane and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated to give an additional demethoxymethylated product. The demethoxymethylated product was dissolved in dichloromethane (800 mL) and *p*-toluenesulfonic acid monohydrate (729 mg, 3.83 mmol) was added. The mixture was refluxed for 3.5 h, cooled to room temperature, washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (dichloromethane) to give **11** as colorless solid (10.2 g, 96%). Recrystallization from dichloromethane–hexane gave colorless needles. Mp 163–164 °C; IR (KBr) 1738, 1519, 1412, 1265, 1225 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.04 (t, J = 7.4 Hz, 2H), 3.83 (s, 3H), 3.84 (s, 3H), 3.88 (s, 3H), 3.95 (s, 3H), 5.14 (t, J = 7.4 Hz, 2H), 5.21 (s, 2H), 6.65 (dd, J = 2.0 and 8.2 Hz, 1H), 6.74 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.92 (s, 1H), 7.30–7.36 (m, 1H), 7.36–7.42 (m, 2H), 7.38 (s, 1H), 7.43–7.48 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.6, 48.7, 52.4, 55.8, 55.9, 56.3, 71.1, 102.7, 105.1, 106.6, 111.3, 112.4, 115.6, 118.5 (q, J = 320 Hz), 120.0, 121.2, 123.2, 127.3, 128.3, 128.8, 129.4, 129.6, 135.9, 145.9, 147.2, 148.0, 149.0, 149.6, 153.8, 158.9. Anal. Calcd for $C_{32}H_{28}F_3NO_{11}S$: C, 55.57; H, 4.08; N, 2.03. Found: C, 55.55; H, 3.99; N, 1.97.

4.2.3. Methyl 7-benzyloxy-3-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dihydro-8-methoxy-1-[4-methoxy-3-(methoxymethoxy)phenyl]-4-oxo-[1]benzopyrano[3,4-*b*]pyrrole-2-carboxylate (13)

According to the procedure described for the preparation of compound **10**, **11** (5.45 g, 7.89 mmol), **12** (3.02 g, 10.3 mmol), and $Pd(PPh_3)_4$ (182 mg, 0.158 mmol) were reacted for 3 h. After chromatographic purification (hexane–ethyl acetate = 1:1–1:2), **13** was obtained as colorless solid (5.24 g, 94%). Recrystallization from dichloromethane–hexane gave colorless powder. Mp 147–148 °C; IR (KBr) 1713, 1516, 1230, 1159 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.08 (t, J = 7.5 Hz, 2H), 3.46 (s, 3H), 3.49 (s, 3H), 3.58 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.94 (s, 3H), 5.10 (dd, J = 6.3 and 8.9 Hz, 2H), 5.16 (s, 2H), 5.22 (s, 2H), 6.55 (s, 1H), 6.75–6.82 (m, 3H), 6.88 (s, 1H), 6.96 (dd, J = 1.9 and 8.3 Hz, 1H), 7.00 (d, J = 8.3 Hz, 1H), 7.13 (d, J = 1.9 Hz, 1H), 7.27–7.32 (m, 1H), 7.33–7.38 (m, 2H), 7.39–7.43 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.9, 48.7, 51.7, 55.6, 55.8, 56.0, 56.1, 56.2, 71.0, 95.7, 102.7, 105.1, 110.0, 111.3, 111.5, 112.4, 117.6, 119.0, 121.2, 124.1, 124.5, 126.9, 126.9, 127.3, 128.1, 128.7, 129.5, 130.6, 136.2, 145.8, 146.3, 146.3, 147.8, 148.2, 149.0, 149.6, 155.1, 161.3. Anal. Calcd for $C_{40}H_{39}NO_{11}$: C, 67.69; H, 5.54; N, 1.97. Found: C, 67.40; H, 5.53; N, 1.92.

4.2.4. 7-Benzyloxy-3-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dihydro-8-methoxy-1-[4-methoxy-3-(methoxymethoxy)phenyl]-4-oxo-[1]benzopyrano[3,4-*b*]pyrrole-2-carboxylic acid (14)

Under an argon atmosphere, a suspension of **13** (3.55 g, 5.00 mmol) in a degassed mixture of 40% aqueous KOH (200 mL) and ethanol (200 mL) was refluxed for 2 h. The solution was cooled to room temperature and concentrated. The pH of the solution was adjusted with acetic acid (225 mL) and the product was extracted with dichloromethane. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was dissolved in dichloromethane (500 mL) and PPTS (1.26 g, 5.00 mmol) was added. The mixture was refluxed for 8 h, cooled to room temperature, and evaporated. The residue was purified by column chromatography (dichloromethane–methanol = 10:1) to give **14** as pale brown solid (2.53 g, 73%). Recrystallization from dichloromethane–diethyl ether hexane gave pale brown powder. Mp 160–200 °C (dec) (sealed capillary); IR (KBr) 1730, 1685, 1437, 1234, 1157 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.07 (t, J = 7.7 Hz, 2H), 3.45 (s, 3H), 3.45 (s, 3H), 3.79 (s, 3H), 3.81 (s, 3H), 3.91 (s, 3H), 5.11 (dd, J = 6.3 and 9.2 Hz, 2H), 5.15 (s, 2H), 5.18 (d, J = 6.5 Hz, 1H), 5.21 (d, J = 6.5 Hz, 1H), 6.49 (s, 1H), 6.70–6.77 (m, 2H), 6.84 (s, 1H), 6.87 (s, 1H), 6.98 (d, J = 8.3 Hz, 1H), 7.02 (dd, J = 1.7 and 8.3 Hz, 1H), 7.19 (d, J = 1.7 Hz, 1H), 7.27–7.32 (m, 1H), 7.32–7.38 (m, 2H), 7.38–7.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.8, 48.8, 55.5, 55.8, 55.9, 56.1, 56.2, 71.0, 95.7, 102.6, 105.0, 109.9, 111.3, 111.7, 112.4, 118.1, 119.2, 121.1, 124.5, 124.6, 125.0, 126.8, 127.0, 127.3, 128.1, 128.7, 130.6, 136.2, 145.7, 146.2, 146.4, 147.8, 148.2, 149.0, 149.8, 155.1, 163.9. Anal. Calcd for $C_{39}H_{37}NO_{11}$: C, 67.33; H, 5.36; N, 2.01. Found: C, 67.07; H, 5.40; N, 1.95.

4.2.5. 7-Benzyloxy-3-[2-(3,4-dimethoxyphenyl)ethyl]-8-methoxy-1-[4-methoxy-3-(methoxymethoxy)phenyl]-[1]benzopyrano[3,4-*b*]pyrrol-4(3*H*)-one (15)

Under an argon atmosphere, a mixture of **14** (1.74 g, 2.50 mmol) and copper(I) oxide (358 mg, 2.50 mmol) in quinoline (25 mL) was heated at 220 °C for 10 min. The mixture was cooled

to room temperature and evaporated. The residue was diluted with dichloromethane and the mixture was passed through a pad of Celite. The filtrate was washed with 1 M HCl, saturated aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (hexane–ethyl acetate = 1:1) to give **15** as pale yellow solid (1.61 g, 99%). Recrystallization from dichloromethane–hexane gave pale yellow powder. Mp 136.5–138 °C; IR (KBr) 1705, 1517, 1260, 1153 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.09 (t, J = 7.0 Hz, 2H), 3.51 (s, 3H), 3.60 (s, 3H), 3.78 (s, 3H), 3.84 (s, 3H), 3.93 (s, 3H), 4.62 (t, J = 7.0 Hz, 2H), 5.18 (s, 2H), 5.23 (s, 2H), 6.62 (d, J = 1.9 Hz, 1H), 6.68 (dd, J = 1.9 and 8.1 Hz, 1H), 6.76 (s, 1H), 6.78 (d, J = 8.1 Hz, 1H), 6.93 (s, 1H), 6.96 (d, J = 8.3 Hz, 1H), 7.02 (dd, J = 2.0 and 8.3 Hz, 1H), 7.11 (s, 1H), 7.18 (d, J = 2.0 Hz, 1H), 7.27–7.33 (m, 1H), 7.33–7.39 (m, 2H), 7.41–7.46 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.8, 50.9, 55.8, 55.9, 56.1, 56.1, 56.4, 71.0, 95.7, 102.9, 105.4, 111.0, 111.4, 111.7, 112.1, 115.0, 118.4, 119.0, 120.9, 123.9, 126.9, 127.1, 127.3, 128.1, 128.7, 130.6, 131.9, 136.4, 146.0, 146.2, 146.4, 147.9, 148.0, 149.0, 149.5, 155.5. Anal. Calcd for $C_{38}H_{37}NO_9$: C, 70.03; H, 5.72; N, 2.15. Found: C, 69.75; H, 5.81; N, 2.11.

4.2.6. 3-Benzyloxy-8,9-dihydro-2,11,12-trimethoxy-14-[4-methoxy-3-(methoxymethoxy)phenyl]-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (16)

Under an argon atmosphere, a solution of PIFA (815 mg, 1.90 mmol) and $BF_3{\cdot}OEt_2$ (480 μL, 3.89 mmol) in dichloromethane (40 mL) was added dropwise to a solution of **15** (1.03 g, 1.59 mmol) in dichloromethane (160 mL) at −40 °C. After being stirred for 1.5 h, the mixture was quenched with 2 M aqueous NH_3 and allowed to warm to room temperature. The product was extracted with dichloromethane and the extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (dichloromethane–ethyl acetate = 20:1) to give **16** as colorless solid (552 mg, 54%). Recrystallization from dichloromethane–diethyl ether gave colorless powder. Mp 223.5–225 °C; IR (KBr) 1706, 1415, 1273, 1171 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.09 (t, J = 6.8 Hz, 2H), 3.39 (s, 3H), 3.46 (s, 3H), 3.49 (s, 3H), 3.87 (s, 3H), 3.95 (s, 3H), 4.67–4.85 (m, 2H), 5.11 (s, 2H), 5.21 (d, J = 6.7 Hz, 1H), 5.25 (d, J = 6.7 Hz, 1H), 6.68 (s, 1H), 6.69 (s, 1H), 6.73 (s, 1H), 6.88 (s, 1H), 7.10 (d, J = 8.2 Hz, 1H), 7.16 (dd, J = 1.9 and 8.2 Hz, 1H), 7.26–7.31 (m, 1H), 7.32–7.37 (m, 2H), 7.33 (d, J = 1.9 Hz, 1H), 7.37–7.42 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.7, 42.4, 55.1, 55.6, 55.9, 56.3, 56.3, 70.9, 95.7, 102.7, 105.0, 108.7, 110.8, 111.0, 112.6, 113.7, 114.6, 119.7, 120.1, 125.4, 126.6, 127.2, 128.0, 128.1, 128.2, 128.6, 135.9, 136.4, 145.8, 146.0, 147.2, 147.5, 147.7, 149.0, 149.9, 155.5. Anal. Calcd for $C_{38}H_{35}NO_9$: C, 70.25; H, 5.43; N, 2.16. Found: C, 69.97; H, 5.39; N, 2.03.

4.2.7. 3-Benzyloxy-2,11,12-trimethoxy-14-[4-methoxy-3-(methoxymethoxy)phenyl]-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (17)

A solution of DDQ (66.3 mg, 0.292 mmol) in dichloromethane (10 mL) was added dropwise to a solution of **16** (182 mg, 0.280 mmol) in dichloromethane (20 mL) at room temperature. After being refluxed for 30 h, the mixture was cooled to room temperature and evaporated. The residue was purified by column chromatography (dichloromethane–ethyl acetate = 20:1) to give **17** as colorless solid (158 mg, 87%). Recrystallization from dichloromethane–diethyl ether gave colorless powder. Mp 240.5–242 °C; IR (KBr) 1703, 1422, 1268, 1173 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.48 (s, 3H), 3.49 (s, 3H), 3.51 (s, 3H), 3.94 (s, 3H), 3.99 (s, 3H), 5.08 (s, 2H), 5.26 (d, J = 6.7 Hz, 1H), 5.29 (d, J = 6.7 Hz, 1H), 6.73 (s, 1H), 6.87 (s, 1H), 6.99 (d, J = 7.4 Hz, 1H), 7.02 (s, 1H), 7.14 (s, 1H), 7.18 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 2.0 and 8.2 Hz, 1H), 7.27–7.32 (m, 1H), 7.33–7.38 (m, 2H), 7.38–7.42 (m, 2H), 7.45 (d, J = 2.0 Hz, 1H), 9.14 (d, J = 7.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.2, 55.5, 55.9, 56.3, 56.4, 70.8, 95.7, 102.6, 105.3, 105.5, 107.4, 107.8, 110.3, 110.7, 112.3, 112.7, 119.1, 120.2, 123.2, 124.8, 126.0, 127.2, 128.0, 128.4, 128.7, 129.3, 134.4, 136.3, 146.0, 146.4, 147.3, 148.5, 149.2, 150.1, 150.2, 155.4. Anal. Calcd for $C_{38}H_{33}NO_9$: C, 70.47; H, 5.14; N, 2.16. Found: C, 70.32; H, 5.11; N, 2.05.

4.2.8. 3-Hydroxy-14-(3-hydroxy-4-methoxyphenyl)-2,11,12-trimethoxy-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]-isoquinolin-6-one (lamellarin α) (1)

Under an argon atmosphere, a heptane solution of BCl_3 (1.0 M, 278 μL, 0.278 mmol) was added dropwise to a solution of **17** (30.0 mg, 0.0463 mmol) in dichloromethane (3.0 mL) at −78 °C. After being stirred for 30 min at this temperature, the reaction mixture was allowed to warm to 0 °C and stirred for an additional 3 h. The mixture was quenched with saturated aqueous $NaHCO_3$ and the precipitate thus formed was collected by filtration, washed with water, and dried under reduced pressure. The residue was purified by column chromatography over Sephadex LH-20 (acetone–methanol = 1:1) to give **1** as pale gray powder (23.3 mg, 98%). Mp > 300 °C (sealed capillary) (lit.[10] Mp > 260 °C); IR (KBr) 3363, 1689, 1430, 1270, 1223, 1163 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.33 (s, 3H), 3.36 (s, 3H), 3.81 (s, 3H), 3.83 (s, 3H), 6.68 (s, 1H), 6.82 (s, 1H), 6.89 (dd, J = 2.0 and 8.2 Hz, 1H), 6.96 (d, J = 2.0 Hz, 1H), 7.07 (s, 1H), 7.12 (d, J = 8.2 Hz, 1H), 7.14 (d, J = 7.4 Hz, 1H), 7.24 (s, 1H), 8.90 (d, J = 7.4 Hz, 1H), 9.44 (br s, 1H), 9.90 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 54.6, 55.2, 55.7, 56.3, 103.9, 104.9, 105.8, 106.7, 108.2, 108.4, 110.8, 112.7, 113.6, 118.3, 118.4, 122.2, 122.4, 124.5, 127.4, 128.8, 133.7, 144.8, 146.5, 147.8, 148.0, 148.1, 149.0, 150.1, 154.5. These spectroscopic data are identical with those previously reported.[10]

4.2.9. 3-[2-(3,4-Dimethoxyphenyl)ethyl]-7-hydroxy-8-methoxy-1-[4-methoxy-3-(methoxymethoxy)phenyl]-[1]benzopyrano[3,4-*b*]pyrrol-4(3*H*)-one (18)

Under a hydrogen atmosphere, a mixture of **15** (100 mg, 0.153 mmol), palladium carbon (Pd: 10%, 20 mg), ethyl acetate (20 mL) was vigorously stirred for 19 h at room temperature. The mixture was passed through a pad of Celite and the filtrate was evaporated. The residue was purified by column chromatography (hexane–ethyl acetate = 1:2) to give **18** as pale brown solid (85.6 mg, 99%). Mp 70–95 °C (dec) (sealed capillary); IR (KBr) 3391, 1708, 1516, 1259, 1154 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.10 (t, J = 7.1 Hz, 2H), 3.51 (s, 3H), 3.61 (s, 3H), 3.79 (s, 3H), 3.85 (s, 3H), 3.93 (s, 3H), 4.63 (t, J = 7.1 Hz, 2H), 5.23 (s, 2H), 5.87 (s, 1H), 6.64 (d, J = 1.9 Hz, 1H), 6.69 (dd, J = 1.9 and 8.1 Hz, 1H), 6.77 (s, 1H), 6.79 (d, J = 8.1 Hz, 1H), 6.97 (d, J = 8.3 Hz, 1H), 6.97 (s, 1H), 7.02 (dd, J = 2.0 and 8.3 Hz, 1H), 7.07 (s, 1H), 7.18 (d, J = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.8, 51.0, 55.8, 55.9, 56.0, 56.1, 56.4, 95.7, 103.5, 104.3, 110.4, 111.4, 111.8, 112.2, 114.9, 118.6, 118.8, 121.0, 124.0, 127.0, 127.3, 130.6, 131.8, 143.4, 145.8, 146.4, 146.6, 147.9, 149.0, 149.5, 155.5; HRFABMS m/z calcd for $C_{31}H_{31}NO_9$ (M^+) 561.1999, found 561.1996.

4.2.10. 3-[2-(3,4-Dimethoxyphenyl)ethyl]-3,4-dihydro-8-methoxy-1-[4-methoxy-3-(methoxymethoxy)phenyl]-4-oxo-[1]benzopyrano[3,4-*b*]pyrrol-7-yl 2,2,2-trichloroethyl sulfate (19)

Under an argon atmosphere, a solution of 2,2,2-trichloroethyl chlorosulfate (38.9 mg, 0.157 mmol) in dichloromethane (3 mL) was added dropwise to a mixture of **18** (67.8 mg, 0.121 mmol), DMAP (14.7 mg, 0.121 mmol), and triethylamine (25.2 μL, 0.181 mmol) in dichloromethane (6 mL) at 0 °C. After being stirred for 1 h at this temperature, the reaction mixture was allowed to

warm to room temperature and stirred for an additional 9 h. The mixture was quenched with water and the product was extracted with dichloromethane. The extract was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography (hexane–ethyl acetate = 1:1) to give **19** as colorless solid (84.0 mg, 90%). Recrystallization from dichloromethane–diethyl ether gave colorless needles. Mp 121.5–122.5 °C; IR (KBr) 1713, 1406, 1261 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.11 (t, J = 7.1 Hz, 2H), 3.51 (s, 3H), 3.60 (s, 3H), 3.81 (s, 3H), 3.86 (s, 3H), 3.94 (s, 3H), 4.66 (t, J = 7.1 Hz, 2H), 4.94 (s, 2H), 5.23 (s, 2H), 6.65 (d, J = 1.8 Hz, 1H), 6.68 (dd, J = 1.8 and 8.1 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 6.82 (s, 1H), 6.98 (d, J = 8.3 Hz, 1H), 7.01 (dd, J = 1.7 and 8.3 Hz, 1H), 7.16 (d, J = 1.7 Hz, 1H), 7.23 (s, 1H), 7.46 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.8, 51.1, 55.8, 55.9, 56.0, 56.1, 56.4, 80.4, 92.4, 95.7, 106.4, 111.3, 111.8, 112.0, 112.4, 115.8, 118.3, 118.4, 119.9, 120.9, 123.9, 125.5, 126.2, 130.2, 132.0, 137.2, 144.7, 146.5, 147.4, 147.9, 149.0, 149.7, 154.6; HRFABMS m/z calcd for $C_{33}H_{32}Cl_3NO_{12}S$ (M^+) 771.0711, found 771.0634.

4.2.11. 3-[2-(3,4-Dimethoxyphenyl)ethyl]-3,4-dihydro-1-(3-hydroxy-4-methoxyphenyl)-8-methoxy-4-oxo-[1]benzopyrano[3,4-*b*]pyrrol-7-yl 2,2,2-trichloroethyl sulfate (20)

To a mixture of **19** (107 mg, 0.139 mmol), dichloromethane (12 mL), and methanol (6.0 mL) was added concd HCl (1.0 mL). The mixture was stirred for 12 h at 30 °C, and evaporated. The precipitate thus formed was collected by filtration, washed with water, and dried under reduced pressure to give **20** as pale brown solid (95.1 mg, 94%). Recrystallization from methanol–water gave pale brown granules. Mp 75–105 °C (dec) (sealed capillary); IR (KBr) 3423, 1719, 1413, 1263 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.03 (t, J = 6.9 Hz, 2H), 3.58 (s, 3H), 3.67 (s, 3H), 3.71 (s, 3H), 3.82 (s, 3H), 4.63 (t, J = 6.9 Hz, 2H), 5.35 (s, 2H), 6.65 (dd, J = 1.7 and 8.2 Hz, 1H), 6.74 (d, J = 1.7 Hz, 1H), 6.82 (dd, J = 2.1 and 8.2 Hz, 1H), 6.83 (d, J = 8.2 Hz, 1H), 6.90 (d, J = 2.1 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 7.36 (s, 1H), 7.41 (s, 1H), 7.67 (s, 1H), 9.35 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.7, 49.7, 55.2, 55.4, 55.7, 55.7, 80.0, 92.8, 106.0, 111.7, 111.9, 112.4, 112.4, 115.1, 116.6, 118.1, 119.7, 120.4, 120.7, 124.0, 125.6, 130.1, 132.8, 136.2, 143.7, 146.5, 147.0, 147.3, 147.4, 148.4, 153.6; HRFABMS m/z calcd for $C_{31}H_{29}Cl_3NO_{11}S$ [$(M+H)^+$] 728.0527, found 728.0543.

4.2.12. Sodium 3-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dihydro-1-(3-hydroxy-4-methoxyphenyl)-8-methoxy-4-oxo-[1]benzopyrano[3,4-*b*]pyrrol-7-yl sulfate (2)

Under an argon atmosphere, a mixture of **20** (50.0 mg, 0.0686 mmol), ammonium formate (25.9 mg, 0.411 mmol), zinc (powder, 13.5 mg, 0.206 mmol), methanol (20 mL), and THF (20 mL) was stirred for 15 h at room temperature and passed through a pad of Celite. The filtrate was evaporated and the residue was purified successively by column chromatography over Silica Gel 60 N (ethyl acetate–methanol = 3:1), column chromatography over Amberlite IRC-50 (Na^+ form) (ethyl acetate–methanol–water = 1:1:1), and column chromatography over Sephadex LH-20 (ethyl acetate–methanol = 1:1) to give **2** as pale yellow powder (38.1 mg, 90%). Mp 170–230 °C (dec) (sealed capillary); IR (KBr) 3424, 1705, 1260, 1049 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.03 (t, J = 7.2 Hz, 2H), 3.50 (s, 3H), 3.67 (s, 3H), 3.71 (s, 3H), 3.81 (s, 3H), 4.61 (t, J = 7.2 Hz, 2H), 6.68 (dd, J = 1.9 and 8.2 Hz, 1H), 6.74 (d, J = 1.9 Hz, 1H), 6.79 (dd, J = 2.1 and 8.2 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 6.91 (d, J = 2.1 Hz, 1H), 7.02 (d, J = 8.2 Hz, 1H), 7.22 (s, 1H), 7.32 (s, 1H), 7.51 (s, 1H), 9.39 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.7, 49.6, 55.2, 55.3, 55.4, 55.6, 105.1, 108.8, 111.7, 112.2, 112.3, 112.4, 114.4, 116.7, 118.8, 120.1, 120.6, 125.3, 126.1, 130.3, 132.5, 142.2, 144.4, 146.5, 146.6, 147.1, 147.3, 148.5, 154.2; HRFABMS m/z calcd for $C_{29}H_{26}NNa_2O_{11}S$ [$(M+Na)^+$] 642.1022, found 642.1015.

4.2.13. 8,9-Dihydro-3-Hydroxy-2,11,12-trimethoxy-14-[4-methoxy-3-(methoxymethoxy)phenyl]-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (21)

According to the procedure described for the preparation of **18**, compound **16** (150 mg, 0.231 mmol) was hydrogenolyzed over palladium carbon (Pd: 10%, 30 mg) for 19 h. After chromatographic purification (dichloromethane–ethyl acetate = 1:1), **21** was obtained as colorless solid (127 mg, 98%). Recrystallization from dichloromethane–diethyl ether gave colorless granules. Mp 225–245 °C (dec) (sealed capillary); IR (KBr) 3107, 1664, 1415, 1273, 1151 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.10 (t, J = 6.9 Hz, 2H), 3.38 (s, 3H), 3.46 (s, 3H), 3.50 (s, 3H), 3.89 (s, 3H), 3.95 (s, 3H), 4.67–4.76 (m, 1H), 4.78–4.87 (m, 1H), 5.20 (d, J = 6.7 Hz, 1H), 5.23 (d, J = 6.7 Hz, 1H), 5.81 (s, 1H), 6.63 (s, 1H), 6.68 (s, 1H), 6.75 (s, 1H), 6.94 (s, 1H), 7.10 (d, J = 8.2 Hz, 1H), 7.16 (dd, J = 1.9 and 8.2 Hz, 1H), 7.32 (d, J = 1.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.7, 42.4, 55.1, 55.6, 56.0, 56.3, 56.3, 95.8, 103.4, 104.2, 108.8, 110.3, 111.1, 112.7, 113.7, 114.4, 120.0, 120.1, 125.6, 126.7, 128.3, 128.3, 136.0, 143.4, 145.6, 146.5, 147.1, 147.5, 149.0, 150.0, 155.6. Anal. Calcd for $C_{31}H_{29}NO_9$: C, 66.54; H, 5.22; N, 2.50. Found: C, 66.48; H, 4.99; N, 2.49.

4.2.14. 8,9-Dihydro-2,11,12-trimethoxy-14-[4-methoxy-3-(methoxymethoxy)phenyl]-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-3-yl 2,2,2-trichloroethyl sulfate (22)

According to the procedure described for the preparation of **19**, compound **21** (85.0 mg, 0.152 mmol) was reacted with 2,2,2-trichloroethyl chlorosulfate (49.0 mg, 0.197 mmol). After chromatographic purification (dichloromethane–ethyl acetate = 20:1), **22** was obtained as colorless solid (116 mg, 99%). Recrystallization from dichloromethane–diethyl ether gave colorless needles. Mp 125–155 °C (dec) (sealed capillary); IR (KBr) 1718, 1487, 1414, 1199 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.13 (t, J = 6.9 Hz, 2H), 3.39 (s, 3H), 3.46 (s, 3H), 3.49 (s, 3H), 3.90 (s, 3H), 3.96 (s, 3H), 4.70–4.79 (m, 1H), 4.80–4.89 (m, 1H), 4.92 (s, 2H), 5.21 (d, J = 6.8 Hz, 1H), 5.23 (d, J = 6.8 Hz, 1H), 6.68 (s, 1H), 6.77 (s, 1H), 6.81 (s, 1H), 7.11 (d, J = 8.2 Hz, 1H), 7.16 (dd, J = 1.9 and 8.2 Hz, 1H), 7.31 (d, J = 1.9 Hz, 1H), 7.42 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.6, 42.6, 55.1, 55.6, 56.0, 56.0, 56.3, 80.4, 92.5, 95.7, 106.2, 108.7, 111.1, 112.3, 112.7, 114.6, 115.3, 118.3, 119.7, 119.7, 125.4, 126.6, 126.7, 127.6, 136.4, 137.1, 144.7, 147.3, 147.3, 147.6, 149.3, 150.2, 154.7. Anal. Calcd for $C_{33}H_{30}Cl_3NO_{12}S$: C, 51.41; H, 3.92; N, 1.82. Found: C, 51.70; H, 3.77; N, 1.71.

4.2.15. 8,9-Dihydro-14-(3-hydroxy-4-methoxyphenyl)-2,11,12-trimethoxy-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-3-yl 2,2,2-trichloroethyl sulfate (23)

According to the procedure described for the preparation of **20**, compound **22** (48.3 mg, 0.0626 mmol) was treated with concd HCl (0.8 mL) in a mixture of dichloromethane (10 mL) and methanol (5 mL) for 20 h to give **23** as colorless solid (42.1 mg, 92%). Recrystallization from acetone–hexane gave colorless powder. Mp 230–240 °C (dec) (sealed capillary); IR (KBr) 3360, 1713, 1413, 1213 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.12 (t, J = 6.7 Hz, 2H), 3.27 (s, 3H), 3.43 (s, 3H), 3.78 (s, 3H), 3.84 (s, 3H), 4.56–4.65 (m, 1H), 4.66–4.75 (m, 1H), 5.35 (s, 2H), 6.71 (s, 1H), 6.90–6.95 (m, 3H), 7.00 (s, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.69 (s, 1H), 9.42 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.4, 54.4, 55.4, 55.5, 55.9, 80.0, 92.8, 105.8, 108.5, 111.6, 111.9, 113.4, 113.7, 115.4, 117.5, 117.8, 118.8, 121.4, 125.3, 126.3, 126.9, 135.6, 136.2, 143.8, 146.9, 147.0, 147.6, 147.8, 149.0, 153.4; HRFABMS m/z calcd for $C_{31}H_{27}Cl_3NO_{11}S$ [$(M+H)^+$] 726.0370, found 726.0374.

4.2.16. Sodium 8,9-dihydro-14-(3-hydroxy-4-methoxyphenyl)-2,11,12-trimethoxy-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-3-yl sulfate (lamellarin U 20-sulfate) (3)

According to the procedure described for the preparation of **2**, compound **23** (100 mg, 0.138 mmol) was reacted with zinc (27.0 mg, 0.413 mmol) and ammonium formate (52.0 mg, 0.825 mmol) to give **3** as pale yellow powder (60.0 mg, 71%). Mp 245–275 °C (dec) (sealed capillary) (lit.[2g] Mp 222–226 °C); IR (KBr) 3446, 1699, 1413, 1275, 1048 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.08 (t, J = 6.7 Hz, 2H), 3.18 (s, 3H), 3.37 (s, 3H), 3.78 (s, 3H), 3.80 (s, 3H), 4.54–4.72 (m, 2H), 6.69 (s, 1H), 6.72 (dd, J = 2.0 and 8.2 Hz, 1H), 6.77 (s, 1H), 6.91 (d, J = 2.0 Hz, 1H), 6.92 (s, 1H), 7.07 (d, J = 8.2 Hz, 1H), 7.48 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.5, 41.9, 54.2, 55.0, 55.4, 55.8, 105.2, 108.4, 108.8, 111.4, 112.3, 112.9, 113.0, 115.2, 117.6, 119.0, 119.7, 126.6, 126.7, 127.0, 135.4, 141.9, 144.4, 146.7, 148.1, 148.7, 149.5, 154.2; HRFABMS m/z calcd for $C_{29}H_{24}NNa_2O_{11}S$ [$(M+Na)^+$] 640.0865, found 640.0851. These spectroscopic data are identical with those previously reported.[2g]

4.2.17. 3-Hydroxy-2,11,12-trimethoxy-14-[4-methoxy-3-(methoxymethoxy)phenyl]-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (24)

According to the procedure described for the preparation of **18**, compound **17** (100 mg, 0.154 mmol) was hydrogenolyzed over palladium carbon (Pd: 10%, 20 mg) for 7 h. After chromatographic purification (dichloromethane–ethyl acetate = 20:1–1:1), **24** was obtained as colorless solid (78.2 mg, 91%). Recrystallization from acetone–hexane gave colorless powder. Mp 230–250 °C (dec) (sealed capillary); IR (KBr) 3308, 1672, 1423, 1268, 1051 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.36 (s, 3H), 3.38 (s, 3H), 3.39 (s, 3H), 3.85 (s, 3H), 3.90 (s, 3H), 5.22 (s, 2H), 6.63 (s, 1H), 6.86 (s, 1H), 7.02 (s, 1H), 7.20 (dd, J = 2.0 and 8.3 Hz, 1H), 7.22 (d, J = 7.4 Hz, 1H), 7.29 (d, J = 8.3 Hz, 1H), 7.33 (s, 1H), 7.35 (d, J = 2.0 Hz, 1H), 8.99 (d, J = 7.4 Hz, 1H), 9.84 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 54.3, 54.8, 55.5, 55.6, 56.0, 94.9, 103.6, 104.5, 105.4, 106.5, 108.0, 108.1, 110.2, 112.5, 113.6, 118.1, 119.8, 122.0, 124.3, 125.5, 127.1, 128.8, 133.5, 144.5, 146.2, 146.6, 147.8, 148.9, 149.8, 150.2, 154.2; HRFABMS m/z calcd for $C_{31}H_{28}NO_9$ [$(M+H)^+$] 558.1764, found 558.1792.

4.2.18. 2,2,2-Trichloroethyl 2,11,12-trimethoxy-14-[4-methoxy-3-(methoxymethoxy)phenyl]-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-3-yl sulfate (25)

According to the procedure described for the preparation of **19**, compound **24** (75.0 mg, 0.135 mmol) was reacted with 2,2,2-trichloroethyl chlorosulfate (43.3 mg, 0.175 mmol). After chromatographic purification (dichloromethane–ethyl acetate = 20:1), **25** was obtained as colorless solid (103 mg, 99%). Recrystallization from dichloromethane–diethyl ether gave colorless needles. Mp 175–195 °C (dec) (sealed capillary); IR (KBr) 1712, 1486, 1415, 1271, 1042 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.48 (s, 3H), 3.50 (s, 3H), 3.51 (s, 3H), 3.99 (s, 3H), 4.00 (s, 3H), 4.92 (s, 2H), 5.26 (d, J = 6.8 Hz, 1H), 5.28 (d, J = 6.8 Hz, 1H), 6.89 (s, 1H), 7.06 (d, J = 7.3 Hz, 1H), 7.08 (s, 1H), 7.14 (s, 1H), 7.20 (d, J = 8.2 Hz, 1H), 7.27 (dd, J = 2.0 and 8.2 Hz, 1H), 7.42 (s, 1H), 7.44 (d, J = 2.0 Hz, 1H), 9.16 (d, J = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.2, 55.6, 56.0, 56.4, 56.4, 80.4, 92.4, 95.7, 105.2, 106.9, 107.5, 108.3, 111.6, 112.4, 112.8, 113.3, 118.0, 119.1, 120.0, 123.0, 124.8, 125.8, 127.6, 127.8, 134.5, 137.8, 145.2, 147.3, 147.4, 149.4, 150.4, 150.4, 154.6; HRFABMS m/z calcd for $C_{33}H_{29}Cl_3NO_{12}S$ [$(M+H)^+$] 768.0476, found 768.0466.

4.2.19. 14-(3-Hydroxy-4-methoxyphenyl)-2,11,12-trimethoxy-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-3-yl 2,2,2-trichloroethyl sulfate (26)

According to the procedure described for the preparation of **20**, compound **25** (94.3 mg, 0.123 mmol) was treated with concd HCl (0.8 mL) in a mixture of dichloromethane (10 mL) and methanol (5 mL) for 20 h to give **26** as colorless solid (87.5 mg, 98%). Recrystallization from acetone–hexane gave colorless powder. Mp 235–245 °C (dec) (sealed capillary); IR (KBr) 3517, 1711, 1487, 1414, 1271 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.37 (s, 3H), 3.47 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 5.37 (s, 2H), 6.94 (s, 1H), 6.96 (dd, J = 1.6 and 8.2 Hz, 1H), 7.02 (d, J = 1.6 Hz, 1H), 7.16 (s, 1H), 7.18 (d, J = 8.2 Hz, 1H), 7.25 (d, J = 7.4 Hz, 1H), 7.32 (s, 1H), 7.69 (s, 1H), 8.92 (d, J = 7.4 Hz, 1H), 9.44 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 54.4, 55.4, 55.5, 56.0, 80.0, 92.8, 104.6, 106.5, 107.3, 108.0, 111.8, 112.0 113.4, 113.4, 117.4, 118.0, 118.1, 121.8, 121.8, 124.2, 126.3, 126.4, 133.4, 137.0, 144.4, 147.0, 147.7, 148.1, 149.0, 150.0, 153.3; HRFABMS m/z calcd for $C_{31}H_{25}Cl_3NO_{11}S$ [$(M+H)^+$] 724.0214, found 724.0207.

4.2.20. Sodium 14-(3-hydroxy-4-methoxyphenyl)-2,11,12-trimethoxy-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-3-yl sulfate (lamellarin α 20-sulfate) (4)

According to the procedure described for the preparation of **2**, compound **26** (60.0 mg, 0.0828 mmol) was reacted with zinc (16.2 mg, 0.248 mmol) and ammonium formate (31.3 mg, 0.497 mmol) to give **4** as pale yellow powder (50.2 mg, 99%). Physical and spectroscopic data of this compound have been reported previously.[12]

4.2.21. 3-Benzyloxy-14-(3-hydroxy-4-methoxyphenyl)-2,11,12-trimethoxy-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (27)

According to the procedure described for the preparation of **20**, compound **17** (100 mg, 0.154 mmol) was treated with concd HCl (1.0 mL) in a mixture of dichloromethane (12 mL) and methanol (6 mL) for 19 h to give **27** as colorless solid (90.1 mg, 97%). Recrystallization from acetone gave colorless powder. Mp 290–300 °C (dec) (sealed capillary); IR (KBr) 3553, 1690, 1430, 1270, 1219, 1168 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.37 (s, 3H), 3.39 (s, 3H), 3.86 (s, 3H), 3.86 (s, 3H), 5.14 (s, 2H), 6.78 (s, 1H), 7.00 (dd, J = 2.1 and 8.0 Hz, 1H), 7.02 (d, J = 2.1 Hz, 1H), 7.16 (s, 1H), 7.17 (s, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.27 (d, J = 7.4 Hz, 1H), 7.32–7.37 (m, 1H), 7.37 (s, 1H), 7.38–7.42 (m, 2H), 7.42–7.47 (m, 2H), 9.01 (d, J = 7.4 Hz, 1H), 9.40 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 54.4, 54.8, 55.5, 56.0, 69.9, 102.2, 104.7, 105.2, 106.7, 108.0, 109.3, 110.9, 112.7, 113.4, 118.1, 118.1, 122.0, 122.0, 124.3, 127.0, 127.8, 127.9, 128.2, 128.4, 133.4, 136.4, 145.4, 145.9, 147.6, 147.9, 148.3, 148.8, 149.9, 154.1; HRFABMS m/z calcd for $C_{36}H_{30}NO_8$ [$(M+H)^+$] 604.1971, found 604.1997.

4.2.22. 5-{3-Benzyloxy-2,11,12-trimethoxy-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-14-yl}-2-methoxyphenyl 2,2,2-trichloroethyl sulfate (28)

According to the procedure described for the preparation of **19**, compound **27** (80.0 mg, 0.133 mmol) was reacted with 2,2,2-trichloroethyl chlorosulfate (42.7 mg, 0.172 mmol). After chromatographic purification (dichloromethane–ethyl acetate = 20:1), **28** was obtained as pale brown solid (106 mg, 98%). Recrystallization from dichloromethane–diethyl ether gave colorless powder. Mp 237.5–239 °C (sealed capillary); IR (KBr) 1703, 1418, 1269, 1167, 1008 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.51 (s, 3H), 3.53 (s, 3H), 3.92 (s, 3H), 4.04 (s, 3H), 5.02 (s, 2H), 5.02 (d, J = 10.7 Hz, 1H), 5.06 (d, J = 10.7 Hz, 1H), 6.55 (s, 1H), 6.78 (s, 1H), 6.97 (s, 1H), 6.98 (d, J = 7.2 Hz, 1H), 7.01 (s, 1H), 7.27–7.32 (m, 1H), 7.32 (d,

J = 8.4 Hz, 1H), 7.33–7.41 (m, 4H), 7.62 (dd, J = 2.2 and 8.4 Hz, 1H), 7.80 (d, J = 2.2 Hz, 1H), 9.09 (d, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 55.3, 55.6, 55.9, 56.6, 70.7, 80.4, 92.5, 102.5, 104.9, 105.1, 107.5, 107.9, 108.6, 109.8, 112.5, 113.7, 118.7, 123.1, 124.8, 126.9, 127.1, 128.1, 128.7, 129.1, 129.3, 132.7, 134.4, 136.2, 139.3, 146.1, 146.3, 148.6, 149.4, 150.2, 151.4, 155.2; HRFABMS m/z calcd for $C_{38}H_{31}Cl_3NO_{11}S$ [$(M+H)^+$] 814.0683, found 814.0709.

4.2.23. 5-{3-Hydroxy-2,11,12-trimethoxy-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-14-yl}-2-methoxyphenyl 2,2,2-trichloroethyl sulfate (29)

Under an argon atmosphere, a heptane solution of BCl_3 (1.0 M, 376 μL, 0.376 mmol) was added dropwise to a solution of **28** (102 mg, 0.125 mmol) in dichloromethane (10 mL) at −78 °C. After being stirred for 30 min at this temperature, the reaction mixture was allowed to warm to 0 °C and stirred for an additional 1 h. The mixture was quenched with saturated aqueous $NaHCO_3$ and the precipitate thus formed was collected by filtration, washed with water, and dried under reduced pressure to give **29** as colorless solid (89.0 mg, 98%). Recrystallization from acetone–hexane gave colorless powder. Mp 285–295 °C (dec) (sealed capillary); IR (KBr) 3310, 1675, 1423, 1267, 1200 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.37 (s, 3H), 3.40 (s, 3H), 3.85 (s, 3H), 4.02 (s, 3H), 5.34 (s, 2H), 6.50 (s, 1H), 6.85 (s, 1H), 6.90 (s, 1H), 7.25 (d, J = 7.4 Hz, 1H), 7.35 (s, 1H), 7.59 (d, J = 8.5 Hz, 1H), 7.68 (dd, J = 2.1 and 8.5 Hz, 1H), 7.87 (d, J = 2.1 Hz, 1H), 9.00 (d, J = 7.4 Hz, 1H), 9.86 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 54.3, 54.8, 55.5, 56.7, 79.8, 92.8, 103.7, 104.2, 104.9, 106.6, 107.8, 108.1, 108.5, 112.7, 114.9, 117.9, 122.0, 124.3, 125.9, 127.6, 128.9, 132.6, 133.6, 138.6, 144.6, 146.1, 147.8, 148.9, 149.9, 151.3, 154.1; HRFABMS m/z calcd for $C_{31}H_{25}Cl_3NO_{11}S$ [$(M+H)^+$] 724.0214, found 724.0241.

4.2.24. Sodium 5-{3-hydroxy-2,11,12-trimethoxy-6-oxo-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-14-yl}-2-methoxyphenyl sulfate (lamellarin α 13-sulfate) (5)

According to the procedure described for the preparation of **2**, compound **29** (60.0 mg, 0.138 mmol) was reacted with zinc (16.2 mg, 0.248 mmol) and ammonium formate (31.3 mg, 0.497 mmol) to give **5** as pale yellow powder (27.8 mg, 55%). Physical and spectroscopic data of this compound have been reported previously.[12]

4.2.25. Disodium salt of 2,11,12-trimethoxy-14-[4-methoxy-3-(sulfooxy)phenyl]-3-(sulfooxy)-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (lamellarin α 13,20-disulfate) (6)

Under an argon atmosphere, a mixture of **1** (40.0 mg, 0.0779 mmol), sulfur trioxide pyridine complex (2.78 g, 17.4 mmol), DMF (2.0 mL), and pyridine (0.5 mL) was stirred for 9 h at 65 °C. After being cooled to room temperature, the reaction mixture was evaporated. To the residue was added saturated aqueous $NaHCO_3$ until the pH of the solution reached about 8 and the solution was evaporated. The residue was suspended in methanol and the mixture was passed through a pad of Celite. The filtrate was evaporated and the residue was purified by column chromatography over Sephadex LH-20 (ethyl acetate–methanol = 1:1) to give **6** as pale yellow powder (44.7 mg, 80%). Physical and spectroscopic data of this compound have been reported previously.[12]

4.2.26. Trisodium salt of 2,12-dimethoxy-14-[4-methoxy-3-(sulfooxy)phenyl]-3,11-bis(sulfooxy)-6*H*-[1]benzopyrano[4′,3′:4,5]pyrrolo[2,1-*a*]isoquinolin-6-one (lamellarin N 8,13,20-trisulfate) (7)

According to the procedure described for the preparation of compound **6**, **30** (38.9 mg, 0.0779 mmol) was reacted with sulfur trioxide pyridine complex (4.17 g, 26.2 mmol). After chromatographic purification over Sephadex LH-20 (methanol), **7** was obtained as pale yellow powder (56.4 mg, 90%). Mp 265–295 °C (dec) (sealed capillary); IR (KBr) 1698, 1433, 1258, 1050 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.39 (s, 6H), 3.88 (s, 3H), 6.75 (s, 1H), 7.16 (s, 1H), 7.24 (dd, J = 1.8 and 8.2 Hz, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.58 (s, 1H), 7.75 (d, J = 1.8 Hz, 1H), 7.91 (s, 1H), 9.07 (d, J = 7.3 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 54.6, 55.0, 56.2, 105.4, 105.6, 107.3, 108.7, 111.6, 111.7, 113.1, 114.1, 117.2, 120.2, 121.9, 123.1, 123.3, 126.1, 126.2, 128.1, 133.4, 142.9, 143.7, 143.7, 144.9, 146.8, 150.4, 150.7, 154.3; HRFABMS m/z calcd for $C_{28}H_{18}NNa_4O_{17}S_3$ [$(M+Na)^+$] 827.9328, found 827.9341.

4.3. Biological assays–general

293T, HeLa, Cos7, and MAGIC 5 cells were cultured in Dulbecco's modified Eagle's medium (Wako) containing 8% fetal bovine serum (FBS) at 37 °C in 5% CO_2. HeLa cells stably expressing CD4 (HeLa/CD4) were constructed as follows. 293T cells were transfected with the MLV gag-pol, CD4-encoding retroviral vector, and VSV-G expression plasmids by TransIT LT-1 reagent (Mirus). The cells were washed 24 h after transfection, and cultured for 24 h in fresh medium. Culture supernatant of the transfected cells was inoculated into HeLa cells. The inoculated cells were selected by puromycin (10 μg/mL). The puromycin-resistant cell pool was utilized in this study.[25]

4.4. Assay of anti-HIV-1 activity

To obtain the LacZ-containing HIV-1 vector, Cos7 cells were transfected with the HXB2 Env, R8.91, and pTY-EFnLacZ expression plasmids using the FuGENE HD transfection reagent (Roche). The transfected cells were washed with medium 24 h after transfection, and continued to be cultured in fresh medium for 24 h. HeLa/CD4 cells were plated into a 6-cm culture dish, and cultured for 24 h. HeLa/CD4 cells were pretreated with the various lamellarin sulfate analogues 5 h before HIV-1 vector infection. Then, HeLa/CD4 cells were inoculated with the culture supernatants of the transfected cells in presence of the lamellarin sulfate analogues. The inoculated cells were stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (Wako) 2 days after inoculation. Blue cells were counted to estimate transduction titer.

4.5. Cytotoxicity assay

HeLa/CD4 cells were cultured onto a 96-well tissues culture plate for 2 days. Cells were treated with various lamellarin sulfate analogues (100 μM) for 24 h. Cells were washed twice with PBS and cultured with medium containing WST-1 reagent (Roche) for 2 h. After the incubation, absorbance of cells was detected at 450 and 650 nm by a micro plate reader (BIO RAD).

4.6. Confocal laser scanning microscopic observation

HeLa/CD4 cells were cultured on four-well culture slides for 24 h. Cells were permeabilizated with methanol (Wako). Permeabilizated or unpermeabilizated cells were cultured with the various lamellarin sulfate analogues (100 μM) for 24 h, washed with PBS and fixed in 4% paraformaldehyde (Wako). Cells were observed using a confocal fluorescence microscope (Leica). The cells were scanned with laser light (400 nm), and fluorescence wavelength from 450 to 500 nm was detected.

4.7. Assay of HIV-1 Env-mediated cell–cell fusion

MAGIC 5 cells were plated onto a 6-cm dish and cultured for 2 days. The MAGIC 5 cells contain the β-galactosidase gene under control of HIV-1 LTR.[26] Cos7 cells were transfected with the mNDK Env expression plasmid by the transfection reagent FuGENE HD. MAGIC 5 cells were pretreated with the various lamellarin sulfate analogues (100 μM) 5 h before coculturing. The transfected cells were washed with medium 24 h after transfection to remove the transfection reagent, and then MAGIC 5 cells were added onto the transfected Cos7 cells in presence of lamellarin sulfate analogues. The Env expression plasmid additionally encodes the tat gene. If these cells fuse, the Tat protein activates the HIV-1 LTR and induces β-galactosidase expression. β-Galactosidase activity of cell lysates was measured 24 h after mixed culture by the high sensitive β-galactosidase activity kit (Stratagene).

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Supplementary data

Supplementary data (experimental data for **8**, **9**, and **12**; UV–Vis and fluorescence spectra of **2**–**7**; ^{1}H and ^{13}C NMR spectra of all compounds synthesized in this work) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.10.030.

References and notes

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