Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3285–3290

BIOORGANIC & MEDICINAL CHEMISTRY

Total Synthesis and Evaluation of Lamellarin α 20-Sulfate Analogues

Christian P. Ridley,[a] M. Venkata Rami Reddy,[a] Genalyn Rocha,[b] Frederic D. Bushman[b] and D. John Faulkner[a,*]

[a]*Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0212, USA*
[b]*Infectious Disease Laboratory, The Salk Institute, 10010 North Torrey Pines Rd., La Jolla, CA, 92037, USA*

Received 6 March 2002; accepted 19 April 2002

Abstract—In order to explore the influence of sulfate groups on the bioactivity profiles of marine alkaloids of the lamellarin class, three such alkaloids, lamellarin α, lamellarin α 13,20-disulfate and lamellarin H, were synthesized and their activities against HIV-1 integrase and cancer cell lines were compared with those of lamellarin α 20-sulfate, which is a selective inhibitor of HIV-1 integrase. Lamellarin α does not inhibit HIV-1 integrase but shows moderate cytotoxicity with good cell line selectivity. Lamellarin α 13,20-disulfate is a moderate inhibitor of both HIV-1 integrase and cancer cell lines. Lamellarin H is a more potent inhibitor of HIV-1 integrase but lacked the specificity required to be medicinally useful.
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Introduction

HIV encodes three enzymes, reverse transcriptase, protease and integrase.[1] Inhibitors of reverse transcriptase and protease form the basis for treatment of HIV-infected people but there is a growing incidence of resistance to some of these drugs.[2] Inhibitors of HIV-1 integrase therefore represent an attractive alternative treatment, particularly because there are no similar enzymes involved in human cellular function.[3] To date, no clinically useful integrase inhibitors have been developed.

The lamellarins are a group of hexacyclic alkaloids that were first isolated from a marine prosobranch mollusc of the genus *Lamellaria*.[4] They were subsequently found in ascidians,[5–11] which are presumed to be the dietary source of the lamellarins found in the mollusc. In 1999, we reported the isolation of lamellarin α 20-sulfate (**1**) (Fig. 1) from an unidentified ascidian collected from the Arabian Sea coast of India. Lamellarin α 20-sulfate (**1**) showed selective inhibition of integrase and inhibited growth of the HIV-1 virus in cell culture ($IC_{50}=8$ μM).[10] Other lamellarin sulfates inhibited integrase less potently but were also inactive against a second enzyme, molluscum contagiosum virus (MCV) topoisomerase, used as a counterscreen.[12] We and others had previously noted that many of the non-sulfated lamellarins were cytotoxic. The most rigorously evaluated member of the series, lamellarin I (**2**), was reported as a potential treatment of some multi-drug resistant cancer cell lines.[13] In the NCI's 60 cell-line panel, lamellarin N (**3**) showed a GI_{50} range over 4 orders of magnitude, with selectivity towards the melanoma cell lines but lamellarin W (**4**) showed no such selectivity.[8] Clearly lacking in these studies was a direct comparison of sulfated and non-sulfated derivatives of a specific lamellarin. For this reason, we first synthesized lamellarin α (**5**) and lamellarin α 13,20-disulfate (**6**) and then synthesized lamellarin H (**7**) due to the observation that many compounds containing the catechol moiety inhibited integrase.[14]

Results and Discussion

Several syntheses of lamellarins have been reported,[15–19] although not all could easily be adapted to produce lamellarin sulfates. At the time we began the synthesis of lamellarin α (**5**), the route described by Banwell and colleagues[17] for the synthesis of lamellarin K (**8**) appeared to provide the most direct entry to the lamellarins. The major difference between lamellarin α (**5**) and lamellarin K (**8**) was the presence of the additional 5,6 double

*Corresponding author. Tel.: +1-858-534-4259; fax: +1-858-534-2997; e-mail: jfaulkner@ucsd.edu

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PII: S0968-0896(02)00237-7

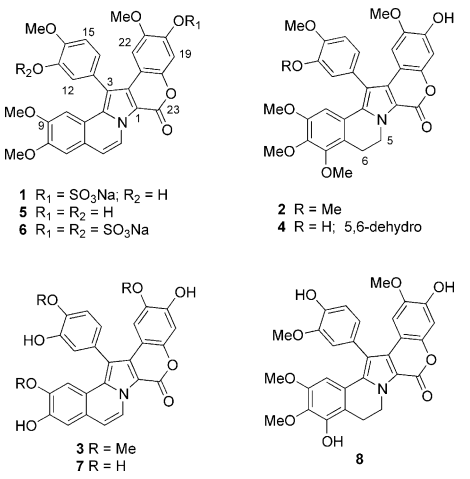
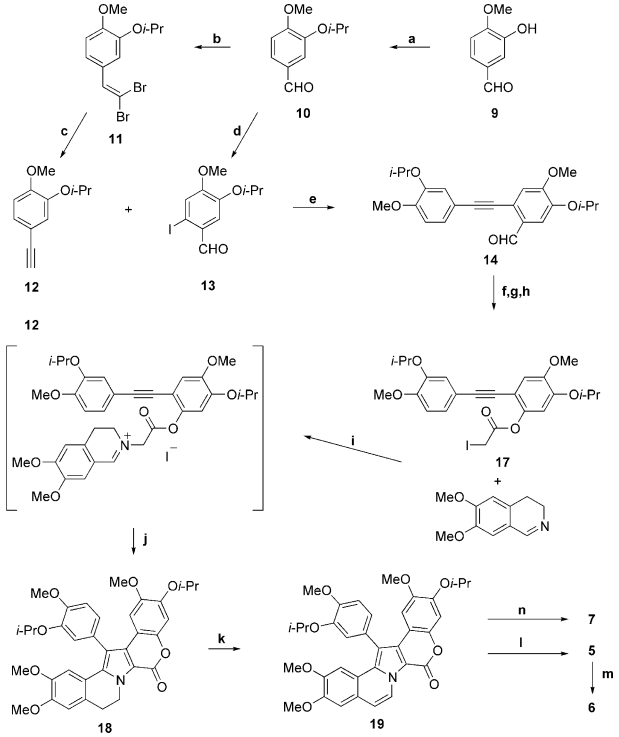


Figure 1.

bond in lamellarin α. Although Banwell and colleagues had reported a more direct synthesis of a model compound containing the 5,6 double bond, we found that it was better to introduce the double bond after forming the cental pyrrole ring. The synthesis of lamellarin α (**5**) (Scheme 1) relied on two coupling reactions. A palladium-mediated coupling of acetylene **12** with 2-iodo-4-methoxy-5-*i*-propoxybenzaldehyde **13** to generate the diarylacetylene **14** was accomplished in 66% yield. A second coupling reaction between the iodoacetate **17** and 3,4-dihydro-6,7-dimethoxyisoquinoline formed an intermediate salt, which on treatment with Hünig's base underwent a [3 + 2] cycloaddition to obtain the pyrrole **18** in 54% yield. All other reactions proceeded smoothly in yields of 80% or better. Using this slightly modified Banwell strategy, lamellarin α (**5**) and lamellarin H (**7**) were each synthesized in 15% overall yield. Using a different route, lamellarin H (**7**) had previously been synthesized by Ishibashi et al. in 15% overall yield.[16]



Scheme 1. (a) *i*-PrBr, K_2CO_3, DMF, rt, 48 h; (b) CBr_4, Zn, PPh_3, CH_2Cl_2, 0–25 °C, 4 h; (c) *n*-BuLi, THF, −78 °C to −25 °C, 2 h; (d) $AgOCOCF_3$, I_2, CH_2Cl_2, reflux, 12 h; (e) $Pd(PPh_3)_4$, CuI, NEt_3, 45 °C, 5 h; (f) MCPBA, $KHCO_3$, CH_2Cl_2, rt, 1 h; (g) NH_3, CH_2Cl_2/MeOH (1:1), rt, 2 h; (h) ICH_2COOH, DCC, DMAP, CH_2Cl_2, rt, 5 h; (i) $ClCH_2CH_2Cl$, rt, 24 h; (j) Hunig's base, reflux, 30 h; (k) DDQ, CH_2Cl_2/EtOH (1:1), reflux, 48 h; (l) BCl_3, CH_2Cl_2, 0 °C, 6 h; (m) DMF-SO_3, DMF/pyridine (4:1), 65 °C, 2 h; (n) BBr_3, $CHCl_3$, 55 °C, 24 h.

Lamellarin α 13,20-disulfate (**6**) was prepared by treatment of lamellarin α (**5**) with the DMF complex of sulfur trioxide in 4:1 DMF/pyridine at 65 °C. Attempts to prepare a mixture of lamellarin α 20-sulfate (**1**) and lamellarin α 13-sulfate by titration of lamellarin α (**5**) with the DMF complex of sulfur trioxide gave only low yields of lamellarin α 13,20-disulfate (**6**). It appears that sulfation of the first phenolic group activates the second phenolic group toward further sulfation, possibly through the intermediacy of a complex of the reagent with the initially-formed sulfate group. It was earlier proposed that the lamellarins exist as racemic mixtures of atropisomers, with the aryl ring at C-3 orthogonal to the plane of the pyrrole ring.[4] We therefore attempted to separate the atropisomers of lamellarin α (**5**) using chiral reagents such as Mosher's reagent or D-(+)-10-camphorsulfonyl chloride but were unsuccessful. The expected products formed but could not be separated by HPLC or on chiral TLC plates.

Lamellarin α (**5**), lamellarin α 13,20-disulfate (**6**) and lamellarin H (**7**) were screened for integrase inhibition, MCV topoisomerase inhibition and cytotoxicity using standard assays described previously (see Table 1).[10,12] The integrase inhibition assay measures the reduction of accumulation of strand transfer products that result from the incubation of purified HIV-1 integrase with target DNA and DNA mimicking one end of the unintegrated viral DNA. Compounds were also screened for inhibition of the type 1B topoisomerase of the molluscum contagiosum virus. This assay, which measures DNA cleavage and religation,[12] is used as a counter-screen to detect non-selective inhibitors. Cytotoxicity toward HeLa cells was measured using MTT as an indicator of cell survival.

Lamellarin α (**5**) showed no inhibition of HIV-1 integrase at concentrations up to 1.6 mM. This result shows that the sulfate group is critical for the HIV-1 integrase activity of lamellarin α 20-sulfate (**1**). This result was

Table 1. Inhibition of HIV-1 integrase (IC_{50}) and MCV topoisomerase (IC_{50}) and cytotoxicity toward HeLa cells (LD_{50}) of lamellarin α (**5**), lamellarin α 20-sulfate (**1**),[10] lamellarin α 13,20-disulfate (**6**) and lamellarin H (**7**)

Compd	Integrase (μM)	MCV (μM)	Cytotoxicity (μM)
5	> 1600	ND	5.1
1	22	> 170	274
6	49	70	29
7	1.3	0.23	5.7

unexpected because other non-sulfated lamellarins inhibited HIV-1 integrase with IC_{50}'s in the low micromolar range.[10] It did however support the idea that the activity of **1** results from the the compound acting as a mimic of the terminal unit of the viral DNA, with the sulfate group binding to a site that normally binds the terminal phosphate of the DNA.

Lamellarin α 13,20-disulfate (**6**, $IC_{50}=49$ μM) was a less effective inhibitor of HIV-1 integrase than was lamellarin α 20-sulfate (**1**, $IC_{50}=22$ μM). It was also much less selective since it inhibited MCV topoisomerase at about the same concentration ($IC_{50}=70$ μM) and is considered a non-selective inhibitor. We propose that the non-selective inhibitors with two or more sulfate groups bind to sites that normally bind to two consecutive phosphate units in intact DNA. This would also explain the 'nuisance' activity of steroidal 2β,3α,6α-trisulfates such as halistanol sulfate and the ibisterol sulfates.[20]

Lamellarin H (**7**, $IC_{50}=1.3$ μM) exhibited very potent inhibition of HIV-1 integrase but unfortunately was even more active in the MCV topoisomerase counterscreen ($IC_{50}=0.23$ μM). It was quite cytotoxic toward HeLa cells ($LD_{50}=5.7$ μM) and was therefore screened, together with lamellarin α (**5**), against a panel of eight human tumor cell lines. Both compounds exhibited good potency and selectivity (**7**: mean $IC_{50}=4$ μM, min/max IC_{50} ratio = 20; **5**: mean $IC_{50}=2.9$ μM, min/max IC_{50} ratio = 10) although the selectivity patterns differed. Since these compounds did not appear to offer any advantages over related lamellarins, further evaluation was not undertaken.

Experimental

General methods

IR and UV spectra were recorded on Perkin Elmer 1600 FTIR and Bio 20 spectrometers, respectively. ^1H and ^{13}C NMR spectra were recorded on Varian Gemini 400 and Inova 300 spectrometers. High resolution Maldi-FTMS data were obtained from the mass spectrometry facility at the Scripps Research Institute, La Jolla (**5**, **6**, **18**, and **19**) while all other high resolution mass measurements were recorded on a VG ZAB mass spectrometer at the UC Riverside Regional Facility. All solvents were redistilled prior to use. Unless otherwise stated, all other commercially available chemicals were used without further purification and all reactions were performed under anhydrous conditions. Products were purified by flash column chromatography using silica gel (200–430 mesh), unless otherwise noted.

Preparation of 3-isopropoxy-4-methoxybenzaldehyde (10)

A suspension of isovanillin (20 g, 130 mmol), isopropyl bromide (19 mL, 203 mmol), anhydrous K_2CO_3 (29.2 g, 211 mmol) in DMF (100 mL) was stirred for 48 h, poured into H_2O (300 mL), and extracted with Et_2O. The ether was then washed with 5% aq NaOH, dried over K_2CO_3, and evaporated to obtain an oil. The oil was purified on a silica column (10% EtOAc/hexanes) to yield isopropyl ether **10** (23.9 g, 94%) as a pale-yellow oil. The spectral data of this compound are identical with those previously reported.[21]

Preparation of 3-isopropoxy-4-methoxyphenylacetylene (12)

A mixture of compound **10** (4 g, 21 mmol), triphenylphosphine (21.6 g, 82 mmol) and zinc dust (5.4 g, 82 mmol) was added to a flask containing carbon tetrabromide (27.3 g, 82 mmol) in CH_2Cl_2 (150 mL) at 0 °C and the reaction mixture was stirred for 4 h at room temperature. The reaction mixture was then poured into pentane (200 mL) and filtered. The filtrate was concentrated and purified on silica gel (25% EtOAc/hexanes) to yield dibromostyrene **11** (6.7 g, 93%) as a pale-yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.24 (d, 1H, $J=2$ Hz), 7.08 (dd, 1H, $J=8.5$, 2 Hz), 6.84 (d, 1H, $J=8.5$ Hz), 4.53 (septet, 1H, $J=6.0$ Hz), 3.86 (s, 3H), 1.38 (d, 6H, $J=6.0$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.5, 146.7, 136.4 (CH), 127.7, 122.1 (CH), 115.3 (CH), 111.3 (CH), 87.0, 71.4 (CH), 55.8 (CH_3), 22.0 ($2CH_3$); HRMS m/z 349.9342 $[M]^+$, calcd for $C_{12}H_{14}{}^{79}Br^{81}BrO_2$, 349.9340.

n-Butyl lithium in hexanes (2.5 M, 120 mL) was added dropwise to a stirred solution of dibromostyrene **11** (6.9 g, 20 mmol) in THF (20 mL) at −78 °C. After 1 h, the solution was allowed to warm to 25 °C and stirred for an additional 1 h. Water (20 mL) was then added and the reaction product was extracted with Et_2O, which was dried over Na_2SO_4 and evaporated to yield an oil. The oil was purified by silica gel chromatography (25% EtOAc/hexanes) to yield alkyne **12** (3 g, 80%) as a white crystalline solid, mp 67–68 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 7.10 (dd, 1H, $J=8.5$, 2 Hz), 7.02 (d, 1H, $J=2$ Hz), 6.80 (d, 1H, $J=8.5$ Hz), 4.52 (sept, 1H, $J=6.5$ Hz), 3.86 (s, 3H), 3.00 (s, 1H), 1.37 (d, 6H, $J=6.5$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.0, 146.6, 125.5 (CH), 119.0 (CH), 114.0, 111.5 (CH), 83.8, 75.4 (CH), 71.6 (CH), 56.0 (CH_3), 22.3 ($2CH_3$); HRMS m/z 190.0998 $[M]^+$, calcd for $C_{12}H_{14}O_2$, 190.0994.

Preparation of 2-iodo-5-isopropoxy-4-methoxybenzaldehyde (13)

In a three-neck round bottom flask with a dropping funnel and a reflux condenser was placed dry silver trifluoroacetate (4.7 g, 21 mmol). The flask was flamed to remove all moisture and **10** (3.6 g, 19 mmol) in CH_2Cl_2 (125 mL) was added. To this stirred suspension a solution of iodine (5.4 g, 21 mmol) in CH_2Cl_2 (300 mL) was added dropwise over a period of 2 h. The solution was refluxed for 12 h, at which point the reaction mixture was filtered, washed with satd. sodium thiosulfate solution, evaporated to dryness, and purified by silica gel chromatography (25% EtOAc/hexanes) to yield iodide **13** as a pale-yellow oil (5.8 g, 95%): ^1H NMR (300 MHz, $CDCl_3$) δ 9.80 (s, 1H), 7.37 (s, 1H), 7.26 (s, 1H), 4.58 (septet, 1H, $J=6$ Hz), 3.88 (s, 3H), 1.33 (d, 6H, $J=6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.7 (CH), 155.5, 147.9, 128.2, 122.2 (CH), 114.0 (CH), 92.4,

71.3 (CH), 56.4 (CH_3), 21.7 ($2CH_3$); HRMS m/z 319.9923 $[M]^+$, calcd for $C_{11}H_{13}O_3I$, 319.9909.

Coupling of acetylene 12 with iodide 13 to form aldehyde 14

To a stirred solution of iodide **13** (692 mg, 2.2 mmol) and triethylamine (30 mL) were added acetylene **12** (411 mg, 2.2 mmol), tetrakis(triphenylphosphine)palladium (25 mg, 0.022 mmol), and copper(I) iodide (8.3 mg, 0.044 mmol). The reaction was stirred for 5 h at 45 °C. Diethyl ether (30 mL) and 0.1 N hydrochloric acid (30 mL) were added, and the organic layer was separated, neutralized with a saturated sodium bicarbonate solution, washed with water, and dried with sodium sulfate. The organic layer was then evaporated to dryness and purified by chromatography on silica (15% EtOAc/hexanes) to yield aldehyde **14** as a yellow solid (555 mg, 66%), mp 109–112 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.49 (s, 1H), 7.41 (s, 1H), 7.21 (dd, 1H, $J=8.5$, 2.5 Hz), 7.06 (d, 1H, $J=2.5$ Hz), 7.03 (s, 1H), 6.84 (d, 1H, $J=8.5$ Hz), 4.70 (septet, 1H, $J=6$ Hz), 4.57 (septet, 1H, $J=6$ Hz), 3.97 (s, 3H), 3.88 (s, 3H), 1.40 (d, 12H, $J=6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.7 (CH), 154.5, 151.3, 147.8, 147.0, 129.8, 125.2 (CH), 121.5, 118.2 (CH), 114.5 (CH), 111.7 (CH), 110.7 (CH), 95.0, 83.4, 71.4 (CH), 71.1 (CH), 56.1 (CH_3), 55.8 (CH_3), 21.9 ($2CH_3$), 21.8 ($2CH_3$); HRMS m/z 382.1777 $[M]^+$, calcd for $C_{23}H_{26}O_5$, 382.1780.

Conversion of aldehyde 14 into iodoacetate 17

A solution of 3-chloroperoxybenzoic acid (104 mg, 0.6 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a vigorously stirred solution of aldehyde **14** (77 mg, 0.2 mmol) and $KHCO_3$ (60 mg, 0.6 mmol) in CH_2Cl_2 (15 mL). After 1 h, the reaction mixture was washed successively with cold water (40 mL), satd. $NaHCO_3$ solution (40 mL) and brine (40 mL), dried over anh. $MgSO_4$ and concentrated to yield a crude product that was further purified by chromatography on silica (25% EtOAC/hexanes) to yield formate 15 (71 mg, 89%) as a white crystalline solid, mp 111–112 °C. IR (NaCl) 2215, 1745 cm^{-1}; UV ($CHCl_3$) 247 nm (ε 21,900), 294 nm (ε 15,700), 346 nm (ε 15,200); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.09 (dd, 1H, $J=8.5$, 1.5 Hz), 7.03 (s, 1H), 7.02 (d, 1H, $J=1.5$ Hz), 6.83 (d, 1H, $J=8.5$ Hz), 6.71 (s, 1H), 4.56 (septet, 1H, $J=6.5$ Hz), 4.55 (septet, 1H, $J=6.5$ Hz), 3.89 (s, 3H), 3.88 (s, 3H), 1.41 (d, 6H, $J=6.5$ Hz), 1.40 (d, 6H, $J=6.5$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0 (CH), 150.8, 148.0, 147.9, 146.7, 144.3, 125.0 (CH), 118.5 (CH), 114.8, 114.8 (CH), 111.6 (CH), 108.7 (CH), 108.4, 93.6, 82.4, 71.9 (CH), 71.7 (CH), 56.5 (CH_3), 56.1 (CH_3), 22.3 ($2CH_3$), 22.2 ($2CH_3$); HRMS m/z 398.1739 $[M]^+$, calcd for $C_{23}H_{26}O_6$, 398.1729.

A solution of ammonia in MeOH (2 M, 0.75 mL) was added to a stirred solution of formate **15** (423.6 mg, 1.06 mmol) in 1:1 CH_2Cl_2/MeOH (5 mL). Anhydrous conditions were not required for this reaction. After 2 h, the reaction mixture was concentrated to dryness, and partitioned between CH_2Cl_2 and water. The organic extract was dried over Na_2SO_4 and the solvent evaporated to yield the phenol **16** (385.7 mg, 98%) as a white crystalline solid, mp 134–137 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (dd, 1H, $J=8$, 2 Hz), 7.10 (d, 1H, $J=2$ Hz), 6.88 (s, 1H), 6.84 (d, 1H, $J=8$ Hz), 6.56 (s, 1H), 5.61 (s, 1H), 4.55 (septet, 2H, $J=6$ Hz), 3.88 (s, 3H), 3.82 (s, 3H), 1.38 (d, 12H, $J=6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.8, 151.1, 149.6, 147.0, 143.9, 125.1 (CH), 118.4 (CH), 114.8, 114.2 (CH), 111.7 (CH), 101.9 (CH), 100.0, 95.2, 81.8, 71.6 (CH), 71.2 (CH), 56.7 (CH_3), 56.0 (CH_3), 22.1 ($2CH_3$), 21.9 ($2CH_3$); HRMS m/z 370.1781 $[M]^+$, calcd for $C_{22}H_{26}O_5$, 370.1780.

Iodoacetic acid (424 mg, 2.28 mmol) and DMAP (12.7 mg, 0.104 mmol) were added to a solution of phenol **16** (767.4 mg, 2.07 mmol) in CH_2Cl_2 (100 mL). A solution of DCC in hexanes (0.68 M, 3.26 mL) was added and the reaction mixture was stirred for 5 h. The precipitated urea was removed by filtration, and the filtrate was concentrated to obtain an oily residue. The oily residue was then purified by chromatography on silica (CH_2Cl_2) to yield iodoacetate **17** (1 g, 90%) as a pale yellow solid, mp 136 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (dd, 1H, $J=8.5$, 2 Hz), 7.06 (d, 1H, $J=2$ Hz), 7.02 (s, 1H), 6.82 (d, 1H, $J=8.5$ Hz), 6.66 (s, 1H), 4.55 (septet, 2H, $J=6$ Hz), 3.96 (s, 2H), 3.87 (s, 6H), 1.40 (d, 6H, $J=6$ Hz), 1.38 (d, 6H, $J=6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.0, 150.9, 148.0, 147.9, 146.8, 144.8, 125.2 (CH), 118.5 (CH), 115.2, 114.9 (CH), 111.6 (CH), 108.5 (CH), 93.3, 82.3, 71.7 (CH), 71.5 (CH), 56.3 (CH_3), 55.9 (CH_3), 22.1 ($2CH_3$), 21.9 ($2CH_3$), 6.6 (CH_2); HRMS m/z 538.0838 $[M]^+$, calcd for $C_{24}H_{27}O_6I$, 538.0852.

Coupling of iodoacetate 17 with 2,3-dihydro-5,6-dimethoxyisoquinoline to obtain lamellarin U diisopropyl ether (18)

3,4-Dihydro-6,7-dimethoxyisoquinoline (212 mg, 1.11 mmol) was added to a solution of iodoacetate 17 (534.4 mg, 0.99 mmol) in CH_2Cl_2 (50 mL) and the resulting mixture was stirred for 21 h at room temperature. *N*,*N*-diisopropylethylamine (142.2 mg, 1.1 mmol) was then added and the solution was refluxed for 30 h. The solvent was then removed under reduced pressure and the resulting oil was subjected to gradient silica gel chromatography (CH_2Cl_2, then EtOAc) to yield lamellarin U diisopropyl ether (**18**, 320.9 mg, 54%) as a white solid, mp 213–214 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (br s, 2H), 7.05 (s, 1H), 6.91 (s, 1H), 6.76 (s, 1H), 6.72 (s, 1H), 6.67 (s, 1H), 4.77 (m, 2H), 4.54 (m, 2H), 3.92 (s, 3H), 3.89 (s, 3H), 3.44 (s, 3H), 3.37 (s, 3H), 3.12 (t, 2H, $J=6.5$ Hz), 1.38 (d, 6H, $J=6$ Hz), 1.34 (d, 6H, $J=6$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 150.3, 149.1, 148.3, 147.6, 147.2, 146.7, 146.1, 135.9, 128.3, 128.2, 126.7, 123.9 (CH), 120.3, 118.4 (CH), 115.0, 113.9, 113.0, 111.2 (CH), 110.7 (CH), 108.9 (CH), 105.3 (CH), 103.9 (CH), 71.7 (2×CH), 56.6 (CH_3), 56.2 (CH_3), 55.8 (CH_3), 55.4 (CH_3), 42.7 (CH_2), 29.0 (CH_2), 22.4 (CH_3), 22.3 (CH_3), 22.2 ($2CH_3$); HRMS m/z 600.2584 $[M+H]^+$, calcd for $C_{35}H_{37}NO_8$, 600.2592.

Conversion of lamellarin U diisopropyl ether (18) into lamellarin α diisopropyl ether (19)

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 391 mg, 1.72 mmol) was added to a solution of lamellarin U

diisopropyl ether (**18**, 260.6 mg, 0.43 mmol) in EtOH/CH_2Cl_2 (1:1, 40 mL) and the reaction mixture was boiled under reflux for 48 h. Evaporation of the solvent followed by chromatography on silica, using a gradient from 100% CH_2Cl_2 to 10% EtOAc in CH_2Cl_2 as eluant, yielded lamellarin α diisopropyl ether (**19**, 244 mg, 93%) as a white solid, mp 211–212 °C. IR (AgCl) 1695, 1460, 1425, 1265, 1220 cm^{-1}; UV ($CDCl_3$) 286 nm (ε 38,600), 367 nm (ε 12,500), 387 nm (ε 16,800); ^1H NMR (400 MHz, DMSO-d_6) δ 9.03 (d, 1H, $J=7.5$ Hz), 7.40 (s, 1H), 7.29 (d, 1H, $J=7.5$ Hz), 7.26 (d, 1H, $J=8.5$ Hz), 7.17 (d, 1H, $J=2$ Hz), 7.13 (dd, 1H, $J=8.5$, 2 Hz), 7.10 (s, 1H), 7.07 (s, 1H), 6.67 (s, 1H), 4.66 (sept., 1H, $J=6$ Hz), 4.56 (sept., 1H, $J=6$ Hz), 3.86 (s, 3H), 3.84 (s, 3H), 3.33 (s, 6H), 1.26 (d, 3H, $J=6$ Hz), 1.25 (d, 3H, $J=6$ Hz), 1.21 (d, 3H, $J=6$ Hz), 1.20 (d, 3H, $J=6$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.7, 149.7, 149.5, 148.4, 147.2, 147.1, 145.6 (2C), 133.2, 128.1, 126.6, 124.0, 123.3, 121.8 (CH), 117.9 (CH), 117.8 (CH), 113.3 (CH), 112.4 (CH), 110.6, 108.8, 107.9 (CH), 106.4, 105.2 (CH), 104.4 (CH), 103.1 (CH), 70.4 (CH), 70.3 (CH), 56.0 (CH_3), 55.5 (CH_3), 55.0 (CH_3), 54.4 (CH_3), 21.8 (CH_3), 21.7 ($3CH_3$); HRMS m/z 620.2241 $[M+Na]^+$, calcd for $C_{35}H_{35}NO_8Na$, 620.2255.

Preparation of lamellarin α (5)

A solution of diisopropyl ether **19** (135.5 mg, 0.22 mmol) in CH_2Cl_2 (20 mL) was cooled to −10 °C and a solution of boron trichloride in CH_2Cl_2 (1 M, 1 mL) was added dropwise. The solution was then stirred at 0 °C for 6 h. The reaction was then quenched with water (20 mL) and the solvent evaporated under reduced pressure. The crude product was purified by chromatography on silica using 1% MeOH in CH_2Cl_2 to yield lamellarin α (**5**, 98.3 mg, 86%) as a white solid, mp >260 °C. IR (AgCl) 3400, 1685, 1425, 1265, 1220, 1160 cm^{-1}; UV ($CDCl_3$): 285 nm (ε 39,200), 366 nm (ε 14,700), 386 nm (ε 20,300); ^1H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H) 9.37 (s, 1H) 9.04 (d, 1H, $J=7$ Hz), 7.40 (s, 1H), 7.28 (d, 1H, $J=7$ Hz), 7.22 (d, 1H, $J=8.5$ Hz), 7.16 (s, 1H), 7.00 (m, 2H), 6.86 (s, 1H), 6.75 (s, 1H), 3.87 (s, 3H), 3.86 (s, 3H), 3.40 (s, 3H), 3.37 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.7, 149.4, 148.4, 147.4, 147.3, 147.1, 145.7, 144.0, 133.0, 128.2, 126.8, 123.9 (CH), 121.8, 121.6 (CH), 117.8, 117.8 (CH), 113.3 (CH), 112.2 (CH), 110.4, 107.8 (CH), 107.8, 106.2, 105.4 (CH), 104.5 (CH), 103.4 (CH), 56.0 (CH_3), 55.5 (CH_3), 55.0 (CH_3), 54.4 (CH_3); HRMS m/z 514.1512 $[M+H]^+$, calcd for $C_{29}H_{24}NO_8$, 514.1496.

Preparation of lamellarin α 13,20-disulfate (6)

DMF-SO_3 complex (1.0 g, 6.5 mmol) was added to a stirred solution of lamellarin α (**5**, 15.2 mg, 0.029 mmol) in DMF/pyridine (4:1, 1 mL). The reaction mixture was stirred for 2 h at 65 °C, after which time the solvent was removed under reduced pressure. The residue was redissolved by addition of satd $NaHCO_3$ solution until solution remained slightly basic. The solution was then loaded onto a C_{18} Sep-Pack cartridge, which was sequentially eluted with H_2O, H_2O/CH_3CN (3:1), H_2O/CH_3CN (1:1) and CH_3CN. The H_2O/CH_3CN (3:1) fraction was further purified by HPLC on a C_{18} column using an elution gradient from H_2O/CH_3CN (4:1) to H_2O/CH_3CN (1:1) to obtain lamellarin α 13,20-disulfate (**6**, 17 mg, 83%) as a pale yellow solid, mp >260 °C (chars). IR (NaCl) 1640, 1440, 1270, 1050 cm^{-1}; UV (MeOH): 203 nm (ε 63,500), 281 nm (ε 38,600), 366 nm (ε 11,500), 386 nm (ε 15,400); ^1H NMR (400 MHz, DMSO-d_6) δ 9.08 (d, 1H, $J=7$ Hz), 7.72 (d, 1H, $J=2$ Hz), 7.55 (s, 1H), 7.42 (s, 1H), 7.33 (d, 1H, $J=7$ Hz), 7.28 (d, 1H, $J=8.5$ Hz), 7.19 (dd, 1H, $J=8.5$, 2 Hz), 7.11(s, 1H), 6.71 (s, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.38 (s, 3H), 3.35 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.7, 150.0, 149.4, 148.5, 146.2, 144.5, 143.5, 142.7, 133.2, 127.8, 125.9, 125.3 (CH), 123.7, 122.7 (CH), 121.7 (CH), 118.0, 113.7 (CH), 112.5 (CH), 111.1, 110.7, 108.3 (CH), 107.8 (CH), 106.6, 105.3 (CH), 104.5 (CH), 56.1 (CH_3), 55.5 (CH_3), 54.9 (CH_3), 54.4 (CH_3); HRMS m/z 740.0101 $[M+Na]^+$, calcd for $C_{29}H_{21}NO_{14}S_2Na_3$, 740.0091.

Preparation of lamellarin H (7)

Boron tribromide (750 uL) was added to a solution of diisopropyl ether **19** (30 mg, 0.05 mmol) in $CHCl_3$ (20 mL) and the reaction mixture was stirred at 55 °C for 24 h. The reaction mixture was cooled to 25 °C and quenched with water (15 mL). The solvent was evaporated and the crude product was purified by chromatography on Sephadex LH-20 using MeOH as eluant to obtain lamellarin H (**7**, 20.1 mg, 88%) as a pale-green solid which turned brown in solution over a period of 24 h. The spectral data of this compound are identical with those previously reported by Lindquist et al.[5]

Acknowledgements

We thank Dr. Craig R. Fairchild, Bristol-Myers Squibb, Princeton, NJ for the eight cell-line bioassay results and Dr. Eric Kantorowski for his advise on synthetic methodology. This research was supported by grants from the National Institutes of Health (CA 49084 and GM 56553).

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