1,3-Dimethyllumazine Derivatives from *Limnatis nilotica*

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Two previously unknown lumazine derivatives, **1** and **2**, have been isolated from the parasitic freshwater leech *Limnatis nilotica.* The structures of the compounds have been elucidated by NMR and unambiguously corroborated by chemical synthesis.

The biochemistry of leeches has traditionally been investigated as an important source of biologically active substances. A classical example is hirudin, a potent thrombin inhibitor isolated more than 40 years ago from the medicinal leech (*Hirudo medicinalis*).[1] Other blood-coagulation modulators,[2] as well as protease inhibitors,[3] have been found in different leeches. For example, potentially important elastase inhibitors have been isolated from *H. medicinalis* and *Hirudo nipponia*.[4,5]

With the goal of finding new pharmacologically interesting substances, we investigated metabolites of *Limnatis nilotica*, a parasitic, freshwater leech with largely unexplored biochemistry.[6] In this paper we report on the isolation, structural elucidation, and synthesis of the novel 1,3-dimethyllumazine derivatives **1** and **2** (Figure 1) isolated from the extract of *L. nilotica*.

A crude H_2O−ethanol extract, prepared from 300 g of cultivated leeches as described,[7] was first chromatographed on a Si gel column using EtOAc/MeCN/H_2O as eluent and then subjected to HPLC purification (linear gradient of MeCN in 0.1% aqueous TFA). Two compounds eluting as sharp peaks with retention times of 20 and 22.4 min were collected to give about 200 μg of each component.

The molecular formulas of fast-eluting **1** ($C_{20}H_{22}N_6O_5$) and slow-eluting **2** ($C_{21}H_{24}N_6O_5$) were obtained from the high-resolution mass spectra [**1**: M^+ 426.1643; **2**: 440.1816]. The identical UV spectra of the two compounds indicated that **1** and **2** were homologues. Furthermore, the UV spectra (absorption maxima at 249 and 337) correlated well with the absorption curve of known lumazine derivatives.[8−10] The ^{1}H NMR spectrum of compound **1**, recorded in D_2O (Table 1) and DMSO-d_6 (Figure 2), were analyzed. A singlet at 9.50 ppm in the ^{1}H NMR spectrum (D_2O) was attributed to the H-7 proton in the pyrazine ring of the lumazine substructure.[8−10] Two three-proton singlets at 3.90 and 3.68 ppm could be assigned to the methyl groups attached to N-1 and N-3 of the lumazine moiety by comparison with known compounds of similar structure.[8−10] Characteristic AA′BB′ patterns at 7.85 and 7.05 ppm were assigned to the aromatic protons of a *para*-substituted benzene ring, while the chemical shifts were similar to those of the aromatic protons in hydroxybenzoic acid derivatives.[11] The presence of a phenolic hydroxyl group was supported by observation in the ^{1}H NMR spectrum (in DMSO-d_6) of a broad resonance of an exchangeable proton at 10 ppm (Figure 2).[11] A correlation of two-proton multiplets at 1.65 and 1.70 ppm (aliphatic CH_2 groups) with doublets of doublets at 3.25 and 3.35 ppm in the 2D COSY spectrum (DMSO-d_6) agreed with a structure in which an alkyl spacer connects two nitrogen atoms. Both nitrogen atoms were assumed to be constituents of amide bonds attached directly to the lumazine and the hydroxyphenyl cores. This

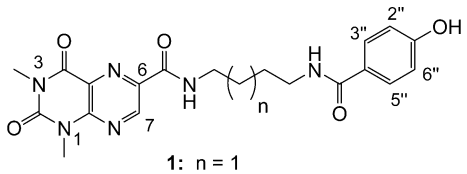

Figure 1. Dimethyllumazine derivatives from *L. nilotica*.

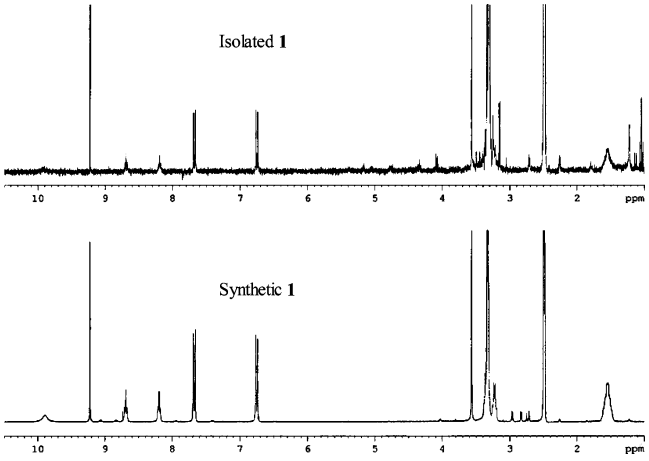

Figure 2. ^{1}H NMR (600 MHz) spectra of the synthetic and isolated compound **1** recorded in DMSO-d_6.

Table 1. ^{1}H NMR Spectral Data of Compounds **1** and **2** (D_2O)

	1	**2**
H-7	9.50 (1H)	9.45 (1H)
H-3″, H-5″	7.85 (2H)	7.74 (2H)
H-2″, H-6″	7.05 (2H)	6.97 (2H)
N1-CH_3	3.90 (3H)	3.90 (3H)
N3-CH_3	3.68 (3H)	3.68 (3H)
CH_2N	3.70 (2H)	3.67 (2H)
	J = 6.6 Hz	J = 6.8 Hz
CH_2N	3.60 (2H)	3.57 (2H)
	J = 6.6	J = 6.8
CH_2	1.75 (4H)	1.65 (4H)
CH_2		1.45 (2H)

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[#] With deep sadness the authors inform the reader that our colleague, Jacques H. van Boom, died on July 31, 2004, at the age of 67.

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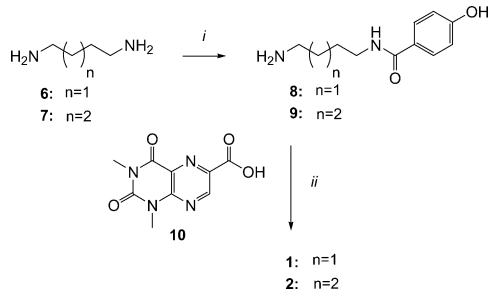
Scheme 1 Chemical Synthesis of Compounds **1** and **2**[a]



[a] *i*. *p*-hydroxybenzoic acid, BOP, DMF, 60 °C; *ii*. BOP, DIPEA, DMF.

assumption was made on the basis of a distinct correlation between the signals at 3.25 and 3.35 ppm with exchangeable proton triplets at 8.19 and 8.65 ppm, respectively (cf. Figure 2). Taken together this evidence is consistent with the structure proposed in Figure 1.

The COSY spectrum (D_2O) of the slow-eluting component (**2**) proved to be nearly identical with the spectrum of **1**, the only significant dissimilarity being an additional two-proton multiplet at 1.45 ppm, which correlated with the multiplet at 1.65 ppm (Table 1), indicating a longer alkyl chain in the latter structure. The spacer length was postulated to be the sole structural difference between **1** and **2**.

To obtain independent proof for the proposed structures, an expeditious synthesis of both compounds was performed as depicted in Scheme 1. First, the commercially available diamines, putrescine (**6**) and cadaverine (**7**) were treated with *p*-hydroxybenzoic acid (*p*-HBA) in the presence of BOP[12] (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) in DMF at 60 °C. Compounds **8** and **9** were isolated as their hydrochloride salts in 22% and 15% yields, respectively, after extensive purification with Si gel and ion exchange chromatography.

The known 1,3-dimethyllumazine-6-carboxylic acid[8] (**10**) was coupled to the free amino group in **8** and **9** in the presence of BOP and DIPEA to give the target compounds **1** and **2** after Si gel column chromatography in 56% and 40% yield, respectively.[13] No protection of the phenolic hydroxyl was necessary due to the mild nature of the BOP activation. The synthetic samples were directly compared with the isolated materials and proved to be indistinguishable as judged by NMR (Figure 2) and RP HPLC analysis (Figure 3). However, the positioning of the acyl substituent at C-6 (not at C-7) of 1,3-dimethyllumazine, which was based on the structures of the known natural lumazine derivatives[9,10] (cf. **3**, **4**, **5**),[14] could not be established with full confidence on the basis of the data presented thus far.

To resolve the last ambiguity in the proposed structures, we prepared regioisomeric compounds **12** and **13** (Scheme 2) containing the *N*-(4-hydroxybenzoyl)-4′-butylaminecarbamoyl moiety attached to the C-7 rather than to the C-6 atom of the lumazine aromatic system. To this end, 1,3-dimethyllumazine-7-carboxylic acid[8] (**11**) was coupled with monoacylated diamines **8** and **9** as described above for isomeric acid **10**. Regioisomers **12** and **13** of natural metabolites **1** and **2** were both obtained in 65% yield and proved to be distinctly different from the natural products. This was established by co-injection of the natural compounds with the synthetic samples of **12** and **13** on a RP HPLC column (Figure 3) as well as ^{1}H NMR spectroscopy of mixtures of **1** with **12** and **2** with **13**.

In conclusion, we have discovered new 1,3-dimethyllumazine-related metabolites[14] of *L. nilotica*. The evaluation

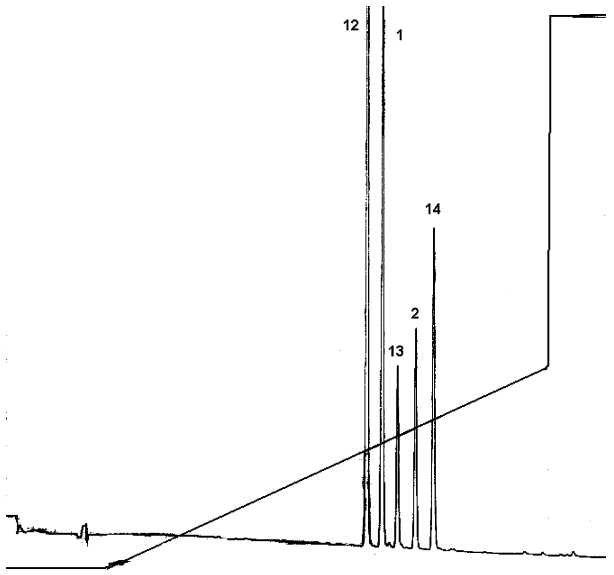
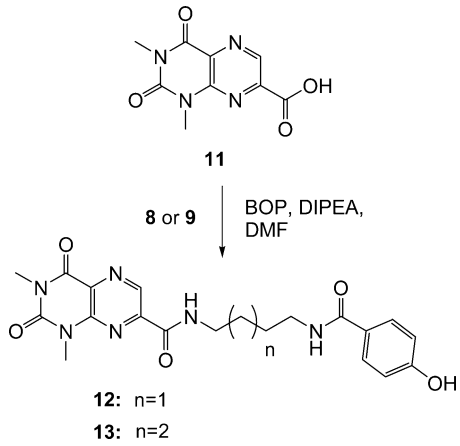


Figure 3. Natural (**1**, **2**) and the nonnatural (**12**−**14**) derivatives co-injected on a Lichrosphere C_{18} HPLC column.

Scheme 2. Preparation of Regioisomers **12** and **13** of the Natural Derivatives **1** and **2**



of pharmacochemical potential of these compounds is now in progress.

Experimental Section

General Experimental Procedures. Dowex 50 W × 8 (200−400 mesh, H^+ form) and Dowex 2 × 8 (200−400 mesh, Cl^- form) were purchased from Fluka. Si gel (0.063−0.2 mm) was from Baker. DMF (p. a., Backer) was stored over 4 Å molecular sieves. MeOH (Biosolve) was of HPLC grade. BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate) was purchased from Neosystem Laboratoire. The other reagents were from Acros Organics. Electrospray mass spectra were recorded using a Perkin-Elmer SCIEX API 165 single quadrupole LC/MS instrument, and the HRMS (SIM mode) spectra were recorded on a TSQ Quantum (Thermo Finnigan) fitted with an accurate mass option, interpolating between PEG-calibration peaks. TLC analysis was performed on Schleicher & Schüll DC Fertigfolien F 1500 LS 254 using CH_2Cl_2/MeOH (9/1, v/v) or CH_2Cl_2/MeOH/NH_4-OH (75/20/5, v/v/v) as the eluent. Analytical RP HPLC was done on a LiChrospher 100 RP-18 column (4.0 × 250 mm, 5 μm particle size, Merck) using a Jasco HPLC pump and a Jacso UV detector with the following buffers: A, 0.1% TFA in 5% aqueous MeCN; B, 0.1% TFA in 90% aqueous MeCN. Elution was performed first isocratically for 2 min (buffer A) and then building up the linear gradient of buffer B in buffer A (0 →

40% B in 30 min), with the retention time (t_R) as specified for each compound.

Biological Material. Crude extract from leeches was obtained as previously described.[7] A total of 300 g of cut heads from frozen *L. nilotica* was extracted first with 94% ethyl alcohol at room temperature (3 × 1.5 L) and subsequently with H_2O (3 × 1.2 L). The combined EtOH and H_2O fractions were directly used in the next step.

Isolation of 1 and 2 from the Crude Extract. The crude extract (obtained from 300 g of leeches) was evaporated to dryness. The residue (0.8 g) was redissolved in MeOH/H_2O, 1/1 (100 mL), Si gel (15 g) was added, and the solvents were evaporated. The resulting powder was applied to a Si gel (30 g) column. Elution with EtOAc/MeCN/H_2O (first from 100/0/0 to 75/25/0 and then from 120/90/10 to 20/130/50) afforded a crude mixture of **1** and **2**. Purification of the latter mixture with semipreparative RP HPLC (Platinum, 10.0 × 250 mm, 5 μm particle size, Alltech) applying an appropriate gradient of MeCN in 0.1% aqueous TFA afforded pure **1** (210 μg) and **2** (196 μg) as white amorphous solids.

1-*N*-(4-Hydroxybenzoyl)-1,4-diaminobutane (8). Putrescine (**6**) (0.88 g, 10 mmol) was dissolved in DMF (50 mL), and a mixture of BOP (1.1 g, 2.5 mmol) and *p*-hydroxybenzoic acid (0.28 g, 2 mmol) in DMF (10 mL) was added. The precipitation of a white crystalline material resulted. The mixture was heated to 60 °C to obtain a clear solution. After 2 h at that temperature the solution was cooled in ice, filtered, and concentrated in high vacuum. The oily, slightly yellowish residue was dissolved in 1 N HCl (50 mL), evaporated to dryness, redissolved in 1 N HCl (20 mL), and applied to a Dowex-H^+ column (4 × 10 cm). The resin was washed with H_2O (300 mL), MeOH (300 mL), and H_2O (400 mL), and then the product was eluted with 25% NH_4OH/H_2O (1/7). Evaporation of the solvents resulted in a white amorphous solid, which was subjected to column chromatography on Si gel. Elution with MeOH/CH_2Cl_2/NH_4OH (2/4/1) gave **8** as a white crystalline solid, which was dried, dissolved in H_2O, and applied to a Dowex-2 (OH^-) column (2 × 10 cm). The column was washed with H_2O (400 mL), 25% NH_4OH (300 mL), and H_2O to neutrality. The product was eluted with 1 N HCl (500 mL) and next with H_2O/MeOH (1/1, 200 mL). Pure **8** was thus obtained as the hydrochloride salt (110 mg, 22%): ^{1}H NMR (300 MHz, MeOD/$CDCl_3$/D_2O, 50/50/5 v/v/v) δ 7.7 (2H, AA′BB′, apparent J = 8.8 Hz, H-3′ H-5′), 6.83 (2H, AA′BB′, apparent J = 8.8 Hz, H-2′ H-6′), 3.37 (2H, t, J = 6.4 Hz, H-1), 2.97 (2H, t, J = 7.1 Hz, H-4), 1.68 (4H, br m, H-2, H-3); ^{13}C NMR (50.1 MHz, CD_3OD) δ 170.8 (C-7′), 162.5 (C-1′), 130.5 (C-3′, C-5′), 124.4 (C-4′), 116.1 (C-2′, C-6′), 40.4, 40.2 (C-1, C-4), 27.0, 25.6 (C-2, C-3); ESIMS *m/z* 209.2 $(M + H)^+$, 231.1 $(M + Na)^+$; HRMS *m/z* 208.1207 (calcd for $C_{11}H_{16}N_2O_2$, 208.1212).

1-*N*-(4-Hydroxybenzoyl)-1,5-diaminopentane (9). Compound **9** was prepared from cadaverine (**7**) (10 mmol, 1.2 mL) and *p*-HBA (0.28 g, 2 mmol) as described for **8** [yield 78 mg (15%) as the hydrochloride salt]: ^{1}H NMR (300 MHz, MeOD/$CDCl_3$/D_2O, 50/50/5 v/v/v) δ 7.57 (2H, AA′BB′, apparent J = 8.8 Hz, H-3′ H-5′), 6.66 (2H, AA′BB′, apparent J = 8.8 Hz, H-2′ H-6′), 3.32 (2H, t, J = 6.4 Hz, H-1), 2.95 (2H, t, J = 7.3 Hz, H-5), 1.60 (4H, br m, H-2 H-4), 1.41 (2H, br m, H-3); ^{13}C NMR (50.1 MHz, $CDCl_3$) δ 170.6 (C-7′), 159.6 (C-1′), 130.1 (C-3′, C-5′), 125.8 (C-4′), 115.8 (C-2′, C-6′), 40.1, 40.0 (C-1, C-5), 28.7, 27.1 (C-2, C-4), 23.7 (C-3); ESIMS *m/z* 223.1 $(M + H)^+$, 245.1 $(M + Na)^+$; HRMS *m/z* 222.1631 (calcd for $C_{12}H_{18}N_2O_2$, 222.1638).

1,3-Dimethyllumazine-6-carboxylic acid (4-(4-hydroxybenzoylamino)butyl)amide (1). 1,3-Dimethyllumazine-6-carboxylic acid[8] (**10**) (20 mg, 85 μmol) was placed in an Eppendorf test tube and was dissolved in a 0.25 M solution of 1-*N*-(4-hydroxybenzoyl)-1,4-diaminobutane (**8**) (0.5 mL, 125 μmol) in DMF. BOP (66 mg, 150 μmol) and DIPEA (90 μL, 0.5 mmol) were added, and the mixture was thoroughly mixed and left for 2 h with occasional shaking. Transfer of the mixture to a 10 mL round-bottom flask and evaporation of the solvent was followed by addition of solid $NaHCO_3$ (14 mg, 170 μmol) and H_2O (2 mL). The slightly brownish precipitate was collected by centrifugation, washed with H_2O (3 mL × 3), and dissolved in MeOH/CH_2Cl_2 (1/2). To this solution was added Si gel (2 g), and the solvents were evaporated. The Si gel was placed on the top of a Si gel column, and the product was eluted with MeOH/CH_2Cl_2 (0/100 → 2/98 → 5/95) to give pure **1** (20 mg, 56%) as a white fluffy solid. A sample of the latter material (5 mg) was crystallized from MeOH/$CHCl_3$/H_2O: mp 256−257 °C; UV (MeOH) λ_{max} 194, 249, 337; ^{1}H NMR (600 MHz, MeOD/$CDCl_3$/D_2O, 50/50/5 v/v/v) δ 9.37 (1H, s, H-7), 7.69 (2H, AA′BB′, apparent J = 8.7 Hz, H-3″ H-5″), 6.82 (2H, AA′BB′, apparent J = 8.7 Hz, H-2″ H-6″), 3.77 (3H, s, Me-1), 3.56 (3H, s, Me-3), 3.52 (2H, t, J = 6.6 Hz, H-1′), 3.44 (2H, t, J = 6.6 Hz, H-4′), 1.75 (4H, br m, H-2′ H-3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 169.5 (C-7 ″), 163.6 (C-9), 161.7 (C-4), 161.0 (C-1″), 151.3 (C-2), 150.1 (C-8a), 148.4 (C-7), 140.8 (C-6), 129.7 (C-3″, C-5″), 126.1 (C-4″), 125.9 (C-4a), 115.7 (C-2″, C-6″), 40.1 (C-4′), 40.0 (C-1′), 30.1 (C-3), 29.4 (C-1), 27.4, 27.3 (C-2′, C-3′); ESIMS *m/z* 427.1 $[M + H]^+$, 449.2 $[M + Na]^+$, (negative mode) 425.1 $[M - H]^-$; HRMS *m/z* 426.1643 (calcd for $C_{20}H_{22}N_6O_5$ 426.1652); *anal.* C 56.18%, H 5.42%, N 19.37%, calcd for $C_{20}H_{22}N_6O_5$ C 56.33%, H 5.20%, N 19.71%; RP HPLC t_R 20 min.

1,3-Dimethyllumazine-7-carboxylic acid (4-(4-hydroxybenzoylamino)butyl)amide (12). 12 was prepared and purified as described for **1** from 1,3-dimethyllumazine-7-carboxylic acid[8] (**11**) (20 mg, 85 μmol). Yield: 23 mg (65%), white solid. An analytical sample was crystallized from MeOH/$CHCl_3$/H_2O to give colorless needles: mp 239−240 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.14 (1H, br t, NH), 9.00 (1H, s H-6), 8.28 (1H, br t, NH), 7.66 (2H, AA′BB′, apparent J = 8.6 Hz, H-3″ H-5″), 6.75 (2H, AA′BB′, apparent J = 8.6 Hz, H-2″ H-6″), 3.62 (3H, s, Me-1), 3.35 (2H, br dd, H-1′), 3.32 (3H, s, Me-3), 3.23 (2H, br dd, H-4′), 1.45 (4H, br m, H-2′ H-3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 166.5 (C-7″), 162.3 (C-9), 160.3 (C-1″), 159.7 (C-4), 150.9 (C-2), 147.1 (C-8a), 146.0 (C-7), 137.7 (C-6), 130.3 (C-4a), 129.4 (C-3″, C-5″), 125.5 (C-4″), 115.1 (C-2″, C-6″), 40.1 (C-4′), 40.0 (C-1′), 29.6 (C-3), 28.9 (C-1), 27.1, 27.0 (C-2′, C-3′); ESIMS *m/z* 427.1 $(M + H)^+$, 449.2 $(M + Na)^+$; (negative mode) 425.1 $(M - H)^-$; HRMS *m/z* 426.1649 (calcd for $C_{20}H_{22}N_6O_5$ 426.1652); *anal.* C 56.13%, H 5.43%, N 19.45%, calcd for $C_{20}H_{22}N_6O_5$ C 56.33%, H 5.20%, N 19.71%; RP HPLC t_R 19 min.

1,3-Dimethyllumazine-6-carboxylic acid (4-(4-hydroxybenzoylamino)pentyl)amide (2). 2 was prepared as described for **1** employing 1-*N*-(4-hydroxybenzoyl)-1,5-diaminopentane (**9**) and 1,3-dimethyllumazine-6-carboxylic acid (**10**) (20 mg, 85 μmol). Purified with column chromatography eluting with MeOH/CH_2Cl_2 (0/100 → 2/98 → 5/95), appropriate fractions were collected and evaporated and the residue was washed with H_2O (2 mL) to give **2** (14 mg, 40%) as a beige amorphous powder: mp 268−270 °C (dec); UV (MeOH) λ_{max} 194, 249, 337; ^{1}H NMR (600 MHz, MeOD/$CDCl_3$/D_2O, 50/50/5 v/v/v) δ 9.36 (1H, s, H-7), 7.62 (2H, AA′BB′, apparent J = 8.8 Hz, H-3″ H-5″), 6.79 (2H, AA′BB′, apparent J = 8.8 Hz, H-2″ H-6″), 3.77 (3H, s, Me-1), 3.56 (3H, s, Me-3), 3.50 (2H, t, J = 6.8 Hz, H-1′), 3.39 (2H, t, J = 6.8 Hz, H-5′), 1.71 (4H, br m, H-2′, H-4′), 1.49 (2H, br m, H-3′); ESIMS *m/z* 441.4 $(M + H)^+$, 463.2 $(M + Na)^+$; (negative mode) 439.2 $(M - H)^-$; *anal.* C 57.03%, H 5.57%, N 18.91%, calcd for $C_{21}H_{24}N_6O_5$ C 57.26%, H 5.49%, N 19.08%; HRMS *m/z* 440.1816 (calcd for $C_{21}H_{24}N_6O_5$ 440.1808); RP HPLC t_R 22.4 min.

1,3-Dimethyllumazine-7-carboxylic acid (4-(4-hydroxybenzoylamino)pentyl)amide (13). 13 was prepared and purified as described for **2**, starting from 1,3-dimethyllumazine-7-carboxylic acid (**11**) (5 mg, 21 μmol). This yielded 4 mg of **13** (43%) as an amorphous off-white powder: mp 262−264 °C (signs of decomposition); ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.90 (1H, brs. OH); 9.11 (1H, br t, NH), 8.99 (1H, s H-6), 8.23 (1H, br t, NH), 7.63 (2H, AA′BB′, apparent J = 8.6 Hz, H-3″ H-5″), 6.73 (2H, AA′BB′, apparent J = 8.6 Hz, H-2″ H-6″), 3.63 (3H, s, Me-1), 3.35 (2H, br dd, H-1′), 3.32 (3H, s, Me-3), 3.23 (2H, br dd, H-5′), 1.55 (4H, br m, H-2′ H-4′); 1.30 (2H, br m, H-3′); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 166.3 (C-7″), 162.0 (C-9), 160.1 (C-1″), 159.6 (C-4), 150.7 (C-2), 147.0 (C-8a), 146.0 (C-7), 137.4 (C-6), 130.2 (C-4a), 129.7 (C-3″, C-5″), 125.7 (C-

4″), 115.3 (C-2″, C-6″), 40.0 (C-5′), 39.9 (C-1′), 29.4 (C-3), 28.6 (C-1), 26.2, 26.8 (C-2′, C-4′); 23.5 (C-3′); ESIMS 441.4 $(M + H)^+$, 463.2 $(M + Na)^+$; (negative mode) 439.2 $(M - H)^-$. HRMS *m/z* 440.1798 (calcd for $C_{21}H_{24}N_6O_5$ 440.1808); *anal.* C 57.02%, H 5.64%, N 18.84%, calcd for $C_{21}H_{24}N_6O_5$ C 57.26%, H 5.49%, N 19.08%; RP HPLC t_R 21 min.

3-Methylaminopyrazine-2,6-dicarboxylic acid 6-((4-(4-hydroxybenzoylamino)butyl)amide) 2-methylamide (14). Compound **1** (5 mg, 11 μmol) was dissolved in 25% NH_4OH (1 mL) and injected on a Q-Sepharose column (16/10, 3 mL/min). The gradient of buffer B (prepared by addition of 25% NH_4OH to a 0.5 M solution of NH_4HCO_3 in 25% NH_4OH/H_2O (3/10) until pH 10.5 was obtained) in buffer A (25% NH_4OH/H_2O, 3/10) was applied (0 → 30% from 0 to 40 mL; 30 → 40% from 40 to 100 mL). The UV-absorbing fractions between 45 and 80 mL were collected, evaporated to dryness, suspended in H_2O, and lyophilized to give **14**, which was further purified using Si gel column chromatography eluting with $MeOH/CH_2Cl_2$ (0/100 → 2/98 → 7/93) to give the title product (2 mg, 44%) as a yellowish solid: ^1H NMR (200 MHz, DMSO-d_6) δ 9.83 (1H, br s, OH), 9.21 (1H, q, $J = 4.7$, NHMe,), 9.06 (1H, q, $J = 5.1$ Hz, NHMe), 8.84 (1H, t, $J = 5.8$ Hz, $NHCH_2$), 8.74 (1H, s, H-5), 8.21 (1H, t, $J = 5.8$ Hz, $NHCH_2$), 7.68 (2H, AA′BB′, apparent $J = 8.8$ Hz, arom.), 6.75 (2H, AA′BB′, apparent $J = 8.8$ Hz, arom.), 2.96 (3H, d, $J = 5.1$ Hz, Me), 2.83 (3H, d, $J = 4.7$ Hz, Me), 1.54 (4H, br s, CH_2CH_2); ESI MS 401.3 $(M + H)^+$, 423.3 $(M + Na)^+$, 439.3 $(M + K)^+$; 823.5 $(2M + Na)^+$; (negative mode) 339.1 $(M - H)^-$, 435.2 $(M + Cl)^-$; HRMS *m/z* 400.1850 (calcd for $C_{21}H_{24}N_6O_5$ 400.1859); RP HPLC t_R 23.6 min.

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References and Notes

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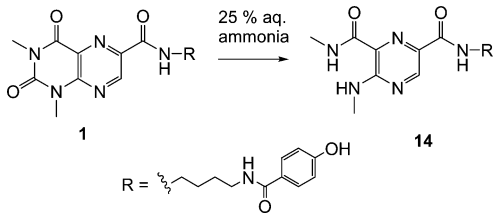
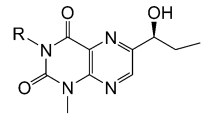
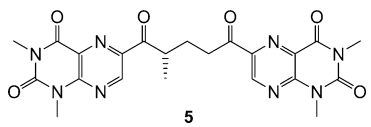
(13) Interestingly the 1,3-dimethyllumazine ring system proved to be unstable under mildly basic conditions. A small amount of product **14** was generated when pure **1** was dissolved in 25% NH_4OH, as could be concluded from ^1H NMR analysis of the material left after evaporation of the solvent. Compound **14** became the major product (44% isolated yield) when we tried to purify compound **1** on a Q-Sepharose column using NH_4OH as the eluent.



(14) To the best of our knowledge, the isolation of lumazine derivatives from leeches has not been reported, although *N*-methylated lumazine metabolites are occasionally found in invertebrates. For example, in 1981 6-substituted methyllumazine analogue **3** was isolated from the marine sponge *Leucetta microraphis.* More recently 1,3-dimethyllumazine derivatives **4** and **5** were found in the marine polychaete *Odontosyllis undecimondta*. See respectively: Cardellina, J. H., II; Meinwald, J. *J. Org. Chem.* **1981**, *46*, 4782−4784. Tanino, H.; Takakura, H.; Kakoi, H.; Okada, K.; Inoue, S. *Heterocycles* **1996**, *42*, 125−128.



3: R = H
4: R = Me



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