65. The Active Centres of Agelastatin A, a Strongly Cytotoxic Alkaloid of the Coral Sea Axinellid Sponge *Agelas dendromorpha*, as Determined by Comparative Bioassays with Semisynthetic Derivatives[1])

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Dedicated to Professor *Mario Piattelli* on the occasion of his 70th birthday

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Agelastatin A (**1**), an unusual alkaloid of the axinellid sponge *Agelas dendromorpha* from the Coral Sea, can be selectively acetylated (→ **7**) or methylated at OH–C(8a) (→ **4**), peracetylated (→ **8**) or permethylated at OH–C(8a), NH(5), and NH(6) (→ **5**), or, finally, subjected to C(9)–C(8a) (→ **14**) or C(5b)–C(8a) β-elimination (→ **11**–**13**), in a regiospecific manner or not, depending on the reaction conditions. Under acidic conditions, compound **12** adds H_2O or MeOH, regioselectively though not *endo*/*exo* stereoselectively, giving *transoid*/*cisoid* mixtures **1**/**18** or **4**/**19**, respectively. Similarly **11** or **13** add MeOH to give mixtures (–)-**2**/**20** or **15**/**16**, respectively. Compound **13** also adds AcOH giving mixture **8**/**17**. The intermediate *cisoid* form obtained on treatment of **21** with H_3O^+ undergoes N(5)–N(6) bridging affording pentacyclic **22** which constitutes a proof for the *cisoid* configuration. From conformational studies, rules are devised that allow assigning the configuration of these compounds from NMR data. *In vitro* comparative cytotoxicity assays of these compounds show that for high cytotoxic activity, such as of **1** *in vivo*, unsubstituted OH–C(8a), H–N(5), H–N(6) moieties are needed in the natural B/D *transoid* configuration.

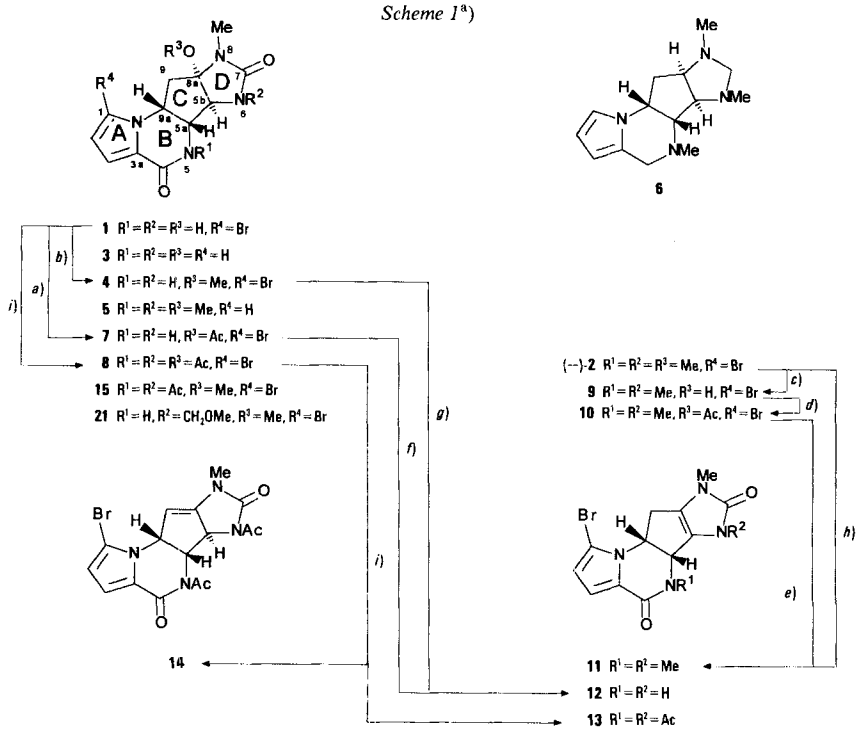
1. Introduction. – Agelastatin A (**1**), recently isolated from the axinellid sponge *Agelas dendromorpha* of deep waters in the Coral Sea [1], has emerged as a most unusual member of alkaloids of the oroidin family for its marked cytotoxicity towards KB tumoural cells *in vitro* and for its unique cyclization pattern [1]. In work designed to assign the absolute configuration of **1** *via* a new application of exciton splitting [2], we obtained *en route* permethylagelastatin A ((–)-**2**), debromoagelastatin A (**3**), 8a-*O*-methylagelastatin A (**4**), debromopermethylagelastatin A (**5**), and the debrominated and deoxygenated product **6** [2].

The problem of a more complete controlled manipulation of agelastatin A (**1**) is addressed here that may help unravelling the active centres responsible for the high cytotoxicity. Moreover, the biological assays carried out *in vivo* too might suggest simplified synthetic targets and perhaps even routes to them.

[1]) Presented in part by *M.D.* at the 'Giornate di Chimica delle Sostanze Naturali', Amalfi, Italy, 29 May to 1 June 1994.

2. Results and Discussion. – 2.1. *Exchange and β-Elimination Processes.* Our first aim was to discriminate further the reactivity at OH–C(8a) from that at NH(5) and NH(6) [2]. Thus, selective OH–C(8a) acylation of **1** by Ac_2O was achieved in pyridine at low temperature to give **7** in 90% yield (*Scheme 1*); when **7** was not isolated but treated with MeOH, **4** was obtained *via* AcO/MeO exchange at C(8a). On raising the temperature by as little as 10°, acylation occurred also at N(5) and/or N(6), giving a mixture of compounds that contained a small amount of peracetylated **8**.

Scheme 1[a])



[a]) Arrows indicate chemical transformations carried out in the present work; for transformations involving the other products, see [1] [2].

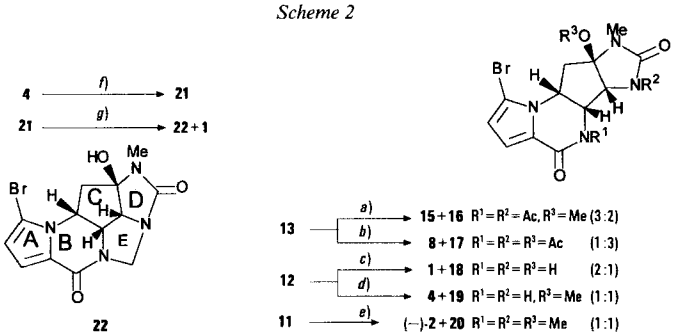
a) Ac_2O/Py 3:1, 0°, overnight, FC; 90%. *b*) 1) Ac_2O/Py 3:1, 0°, overnight; 2) evaporation, MeOH addition, r.t., 5 h; overall 95%. *c*) H_2O/acetone 1:1, *Amberlyst 15*, r.t. 24 h; 100%. *d*) Ac_2O/Py 3:1, r.t., overnight; 100%. *e*) Dry Py, reflux, overnight; 100%. *f*) Dry Py, reflux, 2 h; 100%. *g*) Dry Py, reflux, overnight; 100%. *h*) $CHCl_3$, *Amberlyst 15*, reflux, overnight; 62%. *i*) Ac_2O/Py 3:1, 90°, 24 h, TLC; **8** (34%), **14** (28%), **13** (32%).

Exchange at C(8a) could be reversed: on stirring (–)-**2** in H_2O/acetone 1:1 in the presence of *Amberlyst 15* at room temperature, **9** was obtained, which could be acetylated furnishing **10** (*Scheme 1*). The latter, in warm dry pyridine, gave **11**. On similar treatment, **7** gave **12**. These elimination processes to afford an 1*H*-imidazol-2(3*H*)-one functionality are so favored that **12** or **11** could be obtained by merely heating at reflux overnight **4** in pyridine or (–)-**2** in $CHCl_3$ in the presence of *Amberlyst 15*, respectively.

Interestingly, when the **1**/Ac_2O/pyridine system was heated at 90°, elimination products **13** and **14** were obtained in appreciable yields and could be easily separated from one another and from intermediate peracetylated **8** by prep. TLC (*Scheme 1*). Although the above selective and the latter unselective processes are difficult to compare because of different mechanisms, high temperatures, by favoring the higher activation-energy route, must drive towards unselectivity.

2.2. *Ring-Junction Inversion.* The 1*H*-imidazol-2-(3*H*)-one derivative **13** plays a pivotal role in these transformations. Thus, on treatment with MeOH in the presence of catalytic oxalic acid, **13** gave the 8a-methoxylated derivative **16** with B/D *cisoid* ring arrangement (*Scheme 2*), in a TLC-inseparable mixture with the B/D *transoid* isomer **15** (see *Scheme 1*). In hot Ac_2O containing 4-(dimethylamino)pyridine (DMAP), **13** gave a mixture of the B/D *transoid* peracetylated product **8** and the B/D *cisoid* isomer **17**. Pure **8** could be obtained from this mixture by crystallization from MeCN. The isomeric **14** proved to be much less reactive than **13**, remaining unchanged in MeOH/oxalic acid at room temperature.

Scheme 2



a) MeOH/$(COOH)_2$, r.t., 2 h; **15**/**16** 3:2, overall 85%. *b*) Ac_2O/DMAP, 50°, overnight, TLC; **8**/**17** 1:3, overall 90%. *c*) Acidic H_2O/acetone 1:2, r.t., 15 min, TLC; **1**/**18** 2:1, overall 60%. *d*) MeOH, *Amberlyst 15*, reflux, overnight; **4**/**19** 1:1, overall 80%. *e*) MeOH, $(COOH)_2$, r.t., 1 h; **2**/**20** 1:1, overall 95%. *f*) $CHCl_3/CH_2(OMe)_2$, molecular sieves, *Amberlyst 15*, TLC; 60%. *g*) H_2O, *Amberlyst 15*, reflux, overnight, TLC, **22** (20%) and **1**/**18** 4:1.

High reactivity at the C(8a)=C(5b) bond is also reflected in the behavior of **12** in acidic H_2O or MeOH, yielding B/D *transoid*/*cisoid* mixtures **1**/**18** or **4**/**19**, respectively (*Scheme 2*); the latter could be separated by prep. HPLC into the pure components. Analogously, treatment of **11** with acidic MeOH gave (–)-**2** and its B/D *cisoid* counterpart **20**, which could be easily separated by prep. TLC.

Any suspicion from the above experiments that agelastatin A (**1**) is an artifact of hydration of **12** is ruled out by the failure to detect the B/D *cisoid* isomer **18** during workup of the sponge. In any event, **12** offers itself as an artificial intermediate to arrive at B/D *cisoid* isomers of agelastatin A (**1**) for structure-activity correlation studies.

Attempts at proving the B/D *cisoid* configuration of the above products through spectral measurements were met with difficulties. Thus, the J(5a,5b) coupling for compounds **16**, **17**, and **20** form a different set from that of compounds **18** and **19** thus hindering consistent deductions which are also prevented by the flexibility of these molecules at ring C [2]. Differential NOE experiments on irradiating H–C(5b) while

observing H–C(9a), or *vice versa*, also failed to give clear-cut indications. Unambiguous structural proof had thus to be searched from chemical transformations, following the expectation that, in the B/D *cisoid* form, N(5) and N(6) approaching each other could be interconnected. Such a connection was achieved by treating **4** with acidic $CH_2(OMe)_2$ in dry $CHCl_3$ in the presence of *Amberlyst 15* (*Scheme 2*): intermediate **21** (see *Scheme 1*), heated at reflux in H_2O containing *Amberlyst 15*, gave, along with **1**, pentacyclic **22**, albeit in modest yield, by ring-E closure (*Scheme 2*). This annelation may be rationalized in terms of **21** being prone to two competing processes, deprotection at N(6) and MeOH elimination; the former, after exchange also at C(8a), leads to **1**, while the latter gives a transient 1*H*-imidazol-2(3*H*)-one derivative that can add H_2O to give a B/D *cisoid* intermediate where E-ring closure by N(5) attack occurs, affording **22**.

2.3. *Conformational Analysis.* We have previously shown that agelastatin A and its derivatives are flexible at ring C, a 'crossed' conformer (with orthogonal R^1–N and R^2–N bonds) being in rapid equilibrium with a 'parallel' conformer (with in plane R^1–N and R^2–N bonds) by rotation of the C(5b)–C(5a)–C(9a)–C(9) dihedral angle [2]. Relative abundance of the two conformers in the natural B/D *transoid* series of agelastatin derivatives is mainly determined by steric repulsions between the Br-atom and H_α–C(9) on one side, and the substituents at N(5) and N(6) on the other side; presence of a Br-atom at C(1) and H or Me at N(5) and N(6) favor the 'crossed' conformer.

All compounds belonging to the *cisoid* series examined here have a Br-atom at C(1), and thus the equilibrium position between the two conformers must be determined solely by the bulk of substituents at N(5) and N(6). For a predominance of the 'crossed' conformer over the 'parallel' conformer, these two N-atoms must be brought so close to each other that only H-atoms as substituents may allow it. When so, C(5b)–C(5a)–C(9a)–C(9) and H_α(9)–C(9)–C(9a)–H(9a) dihedral angles take the values of *ca.* +40 and −168° (J(9a,9α) = 11 Hz), respectively, for the 'crossed' conformer, and −40 and −75° (J(9a,9α) small), respectively, for the 'parallel' conformer in both B/D *transoid* and *cisoid* series of compounds. The situation is different for the H(5b)–C(5b)–C(5a)–H(5a) dihedral angle, whose value in the B/D *transoid* series is *ca.* 91° (J(5a,5b) small) or 149° (J(5a,5b) = 8 Hz) for the 'crossed' or the 'parallel' conformer, respectively, while in the B/D *cisoid* series the corresponding values are *ca.* −23° (J(5a,5b) = 6 Hz) and 32° (J(5a,5b) = 8 Hz). Therefore, a compound preferring the 'crossed' conformation (like **1**, **4**, **7**, **9**, **10**, **18**, and **19**) can be unequivocally assigned to the B/D *transoid* or *cisoid* series from knowledge of the J(5a,5b) value, small in the former case or *ca.* 6 Hz in the latter. In contrast, for agelastatin derivatives preferring the 'parallel' conformation (like **15**–**17** or **20**), only a positive NOE between H–C(5a) and H–C(5b) may allow assigning it to the B/D *cisoid* series.

2.4. *Regioselectivity of the Addition Reactions.* Selective acid-catalyzed nucleophilic additions at C(8a) in compounds **11**–**13** is difficult to rationalize. It could be argued that the electron-donating Me group at N(8) in compound **12** stabilizes a positive charge at C(8a), which is thus favored for nucleophilic attack, and that this bias is reinforced in compound **13** by the electron-withdrawing acetyl group at N(6) destabilizing a positive charge at C(5b). Although, on this basis, C(8a) and C(5b) should be equivalent in compound **11**, no product of nucleophilic attack at C(5b) was observed, however. This suggests that electronic factors alone are unable to explain the selectivity of these processes; possibly steric hindrance for attack at C(5b) contributes too. In any event, by

showing how refunctionalization at C(8a) may occur, these observations support the biogenetic hypothesis originally proposed for agelastatin A [1] and may suggest a biogenetic-type total synthesis *via* an intermediate of type **11**–**13**.

2.5. *Structure-Biological Activity Correlations.* Present and previous [2] studies have shown that agelastatin A (**1**) is amenable to a variety of chemical manipulations, such as reductive debromination affording **3**, selective 8a-*O*-acylation or alkylation affording **7** or **4**, respectively, alkylation at N(5) and N(6) giving **9**, C(8a)–C(5b) β-elimination, or unselective C/D ring fusion inversion affording a **1**/**18** mixture. The results of biological assays of these products give a clue as to the centres responsible for high cytotoxicity of agelastatin A against both leukemia and epithelial tumoural cells *in vitro*. Data in the *Table* show that all three unsubstituted functionalities OH–C(8a), NH(5), and NH(6) in the natural C/D ring-fusion configuration as in **1** are needed for optimal cytotoxicity towards leukemia cells, with good resistant index, better than for the antitumoural drug doxorubicin. This leads also to activity by **1** *in vivo* against L1210 leukemia, although only on repeated intraperitoneal (*i.p.*) administration; inactivity by **1** after intravenous (*i.v.*) administration deprives this compound, as such, of therapeutic interest.

Table. *Cytotoxicity Data* (IC_{50} [μg/ml]) *against L1210, L1210/DX, and KB Tumoural Cells* in vitro *for Agelastatin A* (**1**), *Isolated from the Marine Sponge* Agelas dendromorpha, *and for Some of its Semisynthetic Derivatives*

	L1210 IC_{50}	L1210/Dx IC_{50}	RI[a])	KB IC_{50}
1	0.033	0.469	14.0	0.075
(–)-**2**	> 1	> 1	–	> 10
3	0.143	2.11	14.7	–
4	3.970	> 10	–	10
5	> 1	> 1	–	12
6	> 1	> 1	–	–
7	0.729	> 1	–	–
9	> 10	> 10	–	> 10
12	6.71	7.20	1.0	–
18	0.101	2.18	21.4	–
19	3.893	> 10	–	–
Doxorubicin	0.014	0.548	38.8	–

[a]) Resistance index as the ratio between IC_{50} values for L1210/DX cells *vs.* L1210 cells.

The cytotoxic activity decreases dramatically on alkylating or acylating any one of the above three functionalities or by arranging them spatially as in **18**. This holds for both L1210 and KB cells, which differ widely in sensitivity, and also for differently sensitive leukemia cells (*Table*).

Decrease of cytotoxicity on reductive debromination, such as in **3**, is less amenable to interpretation: although it may merely reflect lack of synergism by an 'active' Br-atom, the effect of the lacking Br-atom on the conformational balance offers itself as an alternative explanation.

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Experimental Part

1. *General.* See [2]. Moreover: yields are given on reacted substrate.

2. *Biological Assays.* 2.1. *L1210 and L1210/DX* in vitro *Cytotoxicity Assays.* A growth-inhibition test was performed on both L1210 murine lymphocytic leukemia cell line and the subline resistant to doxorubicin (L1210/Dx); the results were compared with those for doxorubicin (*Pharmacia*) itself. Cells were grown *in vitro* as a stationary suspension culture in RPMI 1640 medium (*Gibco*, Grand Island, NY) supplement with 10% FCS (*Flow Line*, Irvine, UK), 2 mM L-glutamine (*Gibco*), 10 μM 2-mercaptoethanol, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. L1210/Dx Cells were treated at each passage with 100 ng/ml of doxorubicin. Exponentially growing cells were seeded (10^5 cells/ml) and exposed to various concentrations of test compounds immediately after seeding. Inhibition of cell growth was evaluated by counting surviving cells with a *Coulter* counter apparatus after 48 h incubation. The antiproliferative activity of the test compounds was calculated from dose-response curves and expressed as IC_{50} (test compound dose causing 50% inhibition of cell growth in treated cultures relatively to untreated controls).

2.2. *KB* in vitro *Cytotoxicity Assays.* KB Tumor cells were grown *in vitro* as a unicellular film in MEM medium containing *Earle*'s salts and L-glutamine (*Sigma M 0268*) and supplemented with 10% foetal bovine serum (*Sigma F2442*). Exponentially growing cells were seeded after trypsin treatment (10000 cells/ml) in 96 microwells plates and exposed to various concentrations of test compounds 1 h after seeding. The cells were incubated for 3 days, and neutral red was then incorporated. Inhibition of cell growth was evaluated after further 24 h: dead cells were eliminated by rinsing the plates with PBS and hydrolyzing the surviving colored cells with SDS. The *OD* at 540 nm, which is representative of the number of surviving cells, was measured on a *Titertek-Uniskan* apparatus. The antiproliferative activity of the test compounds was calculated from dose-response curves and expressed as IC_{50}.

2.3. In vivo *Assays.* Inbred DBA2 and CD2F1 adult female mice, supplied by *Charles River*, Calco, Italy, were 2–3-months old, weighed between 20 and 24 g, and were kept under standard laboratory conditions. L1210 Murine leukemia (originally obtained from *NCI*) was maintained in DBA2 mice by a serial *i.p.* transplants (10^5 cells/mouse). For experimental studies, CD2F1 mice were inoculated *i.p.* with 10^5 cells/mouse. Agelastatin A (**1**) was administered either *i.p.* or *i.v.* with single or repeated treatments. Agelastatin A activity was evaluated in terms of percentage increase in median survival time in comparison with untreated controls (T/C%). Increase of survival time was 63% at the dose 2.6 mg/kg. Toxicity was evaluated on the basis of the gross autopsy finding and weight loss.

3. *8a-*O*-Acetylagelastatin A* (= *(5a*S*,5b*S*,8a*S*,9a*R*)-8a-(Acetyloxy)-1-bromo-5,5a,5b,6,8,8a,9,9a-octahydro-8-methylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **7**). A soln. of agelastatin A (**1**; 10 mg) in 0.5 ml of Ac_2O/dry pyridine 3:1 was left standing at 0° overnight and then either subjected to FC (*RP-18*, first H_2O, then acetone) yielding **7** (9 mg, 90%) or evaporated to dryness. The residue was dissolved in MeOH and the soln. stirred at r.t. for 5 h) yielding **4** (9.5 mg, 95%). **7**: ^{1}H-NMR (CD_3OD): 6.35 (*d*, *J*(2,3) = 3.9, H–C(2)); 6.93 (*d*, *J*(3,2) = 3.9, H–C(3)); 4.16 (*d*, *J*(5a,9a) = 5.4, H–C(5a)); 4.33 (*s*, H–C(5b)); 2.78 (*s*, Me–N(8)); 2.09 (*s*, AcO); 2.31 (*t*, J_{gem} = 12.9, *J*(9α,9a) = 12.0, H_α–C(9)); 2.83 (*dd*, J_{gem} = 12.9, *J*(9β,9a) = 6.3, H_β–C(9)); 4.65 (*m*, *J*(9a,9β) = 6.3, *J*(9a,9α) = 12.0, *J*(9a,5a) = 5.4, H–C(9a)). ^{13}C-NMR (CD_3OD): 109.00 (*s*, C(1)); 115.51 (*d*, C(2)); 117.77 (*d*, C(3)); 125.70 (*s*, C(3a)); 162.49 (*s*, C(4)); 63.30 (*d*, C(5a)); 65.60 (*d*, C(5b)); 164.00 (*s*, C(7)); 26.69 (*q*, Me–N(8)); 101.49 (*s*, C(8a)); 22.71 (*q*), 172.69 (*s*, AcO); 40.76 (*t*, C(9)); 54.90 (*d*, C(9a)).

4. *5,6-Dimethylagelastatin A* (= *(5a*R*,5b*S*,8a*S*,9a*R*)-1-Bromo-5,5a,5b,6,8,8a,9,9a-octahydro-5,6,8-trimethylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **9**). A mixture of permethylagelastatin A ((–)-**2**; 10 mg), 4 ml of H_2O/acetone 1:1, and *Amberlyst 15* was stirred at r.t. for 24 h and then filtered. The filtrate was evaporated: **9** (quant.). ^{1}H-NMR (CD_3OD): 6.33 (*d*, *J*(2,3) = 4.2, H–C(2)); 6.89 (*d*, *J*(3,2) = 4.2, H–C(3)); 3.18 (*s*, Me–N(5)); 4.23 (br. *d*, *J*(5a,9a) = 6.1, *J*(5a,5b) small, H–C(5a)); 4.30 (br. *s*, *J*(5b,5a) small, H–C(5b)); 2.98 (*s*, Me–N(6)); 2.81 (*s*, Me–N(8)); 2.11 (*t*, J_{gem} = 13.0, *J*(9α,9a) = 12.5, H_α–C(9)); 2.67 (*dd*, J_{gem} = 13.0, *J*(9β,9a) = 6.0, H_β–C(9)); 4.67 (*m*, *J*(9a,9β) = 6.0, *J*(9a,9α) = 12.5, *J*(9a,5a) = 6.1, H–C(9a)). ^{13}C-NMR (CD_3OD): 106.77 (*s*, C(1)); 114.41 (*d*, C(2)); 116.42 (*d*, C(3)); 124.38 (*s*, C(3a)); 160.90 (*s*, C(4)); 31.80 (*q*, Me–N(5)); 65.18 (*d*, C(5a)); 65.88 (*d*, C(5b)); 29.42 (*q*, Me–N(6)); 160.66 (*s*, C(7)); 25.36 (*q*, Me–N(8)); 97.05 (*s*, C(8a)); 40.31 (*t*, C(9)); 53.80 (*d*, C(9a)).

5. *8a-*O*-Acetyl-5,6-dimethylagelastatin A* (= *(5a*R*,5b*S*,8a*S*,9a*R*)-8a-(Acetyloxy)-1-bromo-5,5a,5b,6,8,8a,9,9a-octahydro-5,6,8-trimethylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **10**) *and 5b,8a-Anhydro-5,6-dimethylagelastatin A* (= *(5a*R*,9a*R*)-1-Bromo-5,5a,6,8,9,9a-hexahydro-5,6,8-trimethylimidazo-[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **11**). A soln. of **9** (10 mg) in Ac_2O/dry pyridine 3:1

was stirred at r.t. overnight and then evaporated: **10** (quant.). The latter was dissolved in dry pyridine and heated at 150° overnight: **11** (quant.).

Compound (−)-**2** (8 mg) was heated at reflux in $CHCl_3$ in the presence of *Amberlyst 15* and then subjected to prep. TLC (acetone, collection of the R_f 0.25 band): **11** (5 mg, 62%).

Data of **10**: ^{1}H-NMR ($CDCl_3$): 6.29 (*d*, $J(2,3) = 4.2$, H–C(2)); 6.96 (*d*, $J(3,2) = 4.2$, H–C(3)); 3.14 (*s*, Me–N(5)); 3.99 (*d*, $J(5a,9a) = 6.2$, $J(5a,5b)$ small, H–C(5a)); 4.27 (*s*, $J(5b, 5a)$ small, H–C(5b)); 2.99 (*s*, Me–N(6)); 2.83 (*s*, Me–N(8)); 2.06 (*s*, AcO); 2.33 (*t*, $J_{gem} = 13.0$, $J(9\alpha,9a) = 12.5$, H_α–C(9)); 2.82 (*dd*, $J_{gem} = 13.0$, $J(9\beta,9a) = 6.0$, H_β–C(9)); 4.59 (*m*, $J(9a,9\beta) = 6.0$, $J(9a,9\alpha) = 12.5$, $J(9a,5a) = 6.2$, H–C(9a)).

Data of **11**: ^{1}H-NMR ($CDCl_3$): 6.32 (*d*, $J(2,3) = 4.1$, H–C(2)); 6.94 (*d*, $J(3,2) = 4.1$, H–C(3)); 3.38 (*s*, Me–N(5)); 4.95 (*dd*, $J(5a,9a) = 6.1$, $J(5a,9\beta) = 1.5$, H–C(5a)); 3.05 (*s*, Me–N(6)); 3.25 (*s*, Me–N(8)); 3.31 (*dd*, $J_{gem} = 14.4$, $J(9\alpha,9a) = 7.2$, H_α–C(9)); 2.73 (*ddd*, $J_{gem} = 14.4$, $J(9\beta,9a) = 7.4$, $J(9\beta,5a) = 1.5$, H_β–C(9)); 5.12 (*m*, $J(9a,9\beta) = 7.4$, $J(9a,9\alpha) = 7.2$, $J(9a,5a) = 6.1$, H–C(9a)). ^{13}C-NMR ($CDCl_3$): 104.03 (*s*, C(1)); 113.20 (*d*, C(2)); 114.83 (*d*, C(3)); 123.27 (*s*, C(3a)); 155.98 (*s*, C(4)); 30.22 (*q*, Me–N(5)); 57.45 (*d*, C(5a)); 120.01 (*s*, C(5b)); 29.14 (*s*, Me–N(6)); 154.57 (*s*, C(7)); 29.95 (*q*, Me–N(8)); 125.31 (*s*, C(8a)); 30.96 (*t*, C(9)); 56.50 (*d*, C(9a)). MS: 352, 350 (40, 42, $M^{+\cdot}$); 271 (14), 228, 226 (13, 11); 214 (10); 179 (100). HR-MS: 350.036 ± 0.005 ($C_{14}H_{15}{}^{79}BrN_4O_2^+$, calc. 350.038).

6. *5b,8a-Anhydroagelastatin A* (= *(5a*R*,9a*R*)-1-Bromo-5,5a,6,8,9,9a-hexahydro-8-methylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **12**). A soln. of **7** (20 mg) in dry pyridine was heated at 150° for 2 h and then evaporated: **12** (quant.). Similarly **4** was transformed to **12**. ^{1}H-NMR (CD_3OD): 6.39 (*d*, $J(2,3) = 4.1$, H–C(2)); 6.90 (*d*, $J(3,2) = 4.1$, H–C(3)); 5.11 (*dd*, $J(5a,9a) = 7.2$, $J(5a,9\beta) = 1.8$, H–C(5a)); 3.22 (*s*, Me–N(8)); 3.48 (*dd*, $J_{gem} = 14.4$, $J(9\alpha,9a) = 7.2$, H_α–C(9)); 2.63 (*ddd*, $J_{gem} = 14.4$, $J(9\beta,9a) = 7.2$, $J(9\beta,5a) = 1.8$, H_β–C(9)); 5.34 (*q*, $J(9a,9\beta) = J(9a,9\alpha) = J(9a,5a) = 7.2$, H–C(9a)). ^{13}C-NMR ($CD_3OD$): 108.62 (*s*, C(1)); 115.65 (*d*, C(2)); 117.41 (*d*, C(3)); 125.42 (*s*, C(3a)); 161.45 (*s*, C(4)); 58.52 (*d*, C(5a)); 121.71 (*s*, C(5b)); 159.92 (*s*, C(7)); 30.49 (*q*, Me–N(8)); 128.88 (*s*, C(8a)); 33.77 (*t*, C(9)); 54.55 (*d*, C(9a)).

7. *Compounds* **8**, **13**, *and* **14**. A soln. of **1** (56 mg) in Ac_2O/dry pyridine 3:1 was heated at 90° for 24 h and then evaporated. The residue was subjected to prep. TLC (CH_2Cl_2/AcOEt 1:1): **13** (R_f 0.77; 18 mg, 32%), **8** (R_f 0.60; 19 mg, 34%), and **14** (R_f 0.45; 16 mg, 28%).

*5,6,8a-*O*-Triacetylagelastatin A* (= *(5a*R*,5b*S*,8a*S*,9a*R*)-5,6-Diacetyl-8a-(acetyloxy)-1-bromo-5,5a,5b,6,8,8a,9,9a-octahydro-8-methylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **8**). ^{1}H-NMR (CD_3OD): 6.42 (*d*, $J(2,3) = 4.2$, H–C(2)); 7.19 (*d*, $J(3,2) = 4.2$, H–C(3)); 2.45 (*s*, AcO–N(5)); 5.52 (*dd*, $J(5a,9a) = 5.1$, $J(5a,5b) = 8.5$, H–C(5a)); 4.75 (br. *d*, $J(5b,5a) = 8.5$, $J(5b,9\alpha) = 0.9$, H–C(5b)); 2.58 (*s*, AcO–N(6)); 2.83 (*s*, Me–N(8)); 1.81 (*s*, AcOO–C(8a)); 4.01 (*td*, $J_{gem} = 15.8$, $J(9\alpha,9a) = J(9\alpha,5b) = 0.9$, H_α–C(9)); 2.42 (*dd*, $J_{gem} = 15.8$, $J(9\beta,9a) = 5.1$, H_β–C(9)); 4.70 (*t*, $J(9a,5a) = J(9a,9\beta) = 5.1$, $J(9a,9\alpha) = 0.9$, H–C(9a)). ^{13}C-NMR (CD_3OD): 106.46 (*s*, C(1)); 115.94 (*d*, C(2)); 118.94 (*d*, C(3)); 126.40 (*s*, C(3a)); 155.59 (*s*, C(4)); 23.38 (*q*), 170.23 (*s*, AcO–N(5)); 58.87 (*d*, C(5a)); 64.48 (*d*, C(5b)); 26.50 (*q*); 172.13 (*s*, AcO–N(6)); 153.15 (*s*, C(7)); 25.42 (*q*, Me–N(8)); 93.61 (*s*, C(8a)); 20.74 (*q*), 169.04 (*s*, *Ac*OO–C(8a)); 40.32 (*t*, C(9)); 55.96 (*d*, C(9a)). MS: 468, 466 (4, 4, $M^{+\cdot}$); 408, 406 (4, 4); 366, 364 (8, 9); 324, 322 (4, 5); 243 (12); 193 (35); 43 (100). HR-MS: 466.048 ± 0.006 ($C_{18}H_{19}{}^{79}BrN_4O_6^+$, calc. 466.049).

5,6-Diacetyl-5b,8a-anhydroagelastatin A (= *(5a*R*,9a*R*)-5,6-Diacetyl-1-bromo-5,5a,6,8,9,9a-hexahydro-8-methylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **13**). ^{1}H-NMR ($CDCl_3$): 6.43 (*d*, $J(2,3) = 4.2$, H–C(2)); 7.10 (*d*, $J(3,2) = 4.2$, H–C(3)); 2.55 (*s*, AcO–N(5)); 6.76 (*ddd*, $J(5a,9a) = 6.5$, $J(5a,9\alpha) = 2.4$, $J(5a,9\beta) = 1.5$, H–C(5a)); 2.60 (*s*, AcO–N(6)); 3.15 (*s*, Me–N(8)); 3.19 (*ddd*, $J_{gem} = 16.4$, $J(9\beta,9a) = 6.0$, $J(9\beta,5a) = 2.4$, H_β–C(9)); 3.33 (*ddd*, $J_{gem} = 16.4$, $J(9\alpha,9a) = 0.9$, $J(9\alpha,5a) = 1.5$, H_α–C(9)); 5.18 (*td*, $J(9a,5a) = 6.5$, $J(9a,9\beta) = 6.0$, $J(9a,9\alpha) = 0.9$, H–C(9a)). ^{13}C-NMR ($CDCl_3$): 106.56 (*s*, C(1)); 115.54 (*d*, C(2)); 118.02 (*d*, C(3)); 128.06 (*s*, C(3a)); 157.32 (*s*, C(4)); 24.51 (*q*), 168.49 (*s*, AcO–N(5)); 53.70 (*d*, C(5a)); 117.46 (*s*, C(5b)); 26.09 (*q*), 172.12 (*s*, AcO–N(6)); 154.36 (*s*, C(7)); 28.72 (*q*, Me–N(8)); 126.90 (*s*, C(8a)); 32.35 (*t*, C(9)); 57.06 (*d*, C(9a)).

5,6-Diacetyl-8a,9-anhydroagelastatin A (= *(5a*R*,5b*S*,9a*R*)-5,6-Diacetyl-1-bromo-5,5a,5b,6,8,9a-hexahydro-8-methylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **14**). ^{1}H-NMR ($CDCl_3$): 6.37 (*d*, $J(2,3) = 4.2$, H–C(2)); 7.13 (*d*, $J(3,2) = 4.2$, H–C(3)); 2.45 (*s*, AcO–N(5)); 5.56 (*dd*, $J(5a,9a) = 5.7$, $J(5a,5b) = 8.1$, H–C(5a)); 4.90 (*ddd*, $J(5b,5a) = 8.1$, $J(5b,9) = 2.7$, $J(5b,9a) = 1.0$, H–C(5b)); 2.63 (*s*, AcO–N(6)); 3.07 (*s*, Me–N(8)); 5.78 (*dd*, $J(9,9a) = 3.6$, $J(9,5b) = 2.7$, H–C(9)); 5.14 (*ddd*, $J(9a,5a) = 5.7$, $J(9a,9) = 3.6$, $J(9a,5b) = 1.0$, H–C(9a)). ^{13}C-NMR ($CDCl_3$): 107.33 (*s*, C(1)); 115.15 (*d*, C(2)); 117.83 (*d*, C(3)); 125.72 (*s*, C(3a)); 156.26 (*s*, C(4)); 23.87 (*q*), 170.53 (*s*, AcO–N(5)); 60.37 (*d*, C(5a)); 62.32 (*d*, C(5b)); 26.93 (*q*), 172.69 (*s*, AcO–N(6)); 155.16 (*s*, C(7)); 28.40 (*q*, Me–N(8)); 145.45 (*s*, C(8a)); 94.87 (*d*, C(9)); 61.22 (*d*, C(9a)).

8. *5,6-Diacetyl-8a-*O*-methylagelastatin A* (= *(5aR,5bS,8aS,9aR)-5,6-Diacetyl-1-bromo-5,5a,5b,6,8,8a,9,9a-octahydro-8a-methoxy-8-methylimidazo[4',5':4,5]cyclopenta[1,2-e]pyrrolo[1,2-a]pyrazine-4,6-dione*; **15**) *and 5,6-Diacetyl-8a-*O*-methyl-5b,8a-diepiagelastatin A* (**16**). A soln. of **13** (5 mg) in 1 ml of MeOH containing catalytic $(COOH)_2$ was stirred at r.t. for 2 h and then evaporated: **15/16** 3:2. Yield 85%. MS: 440, 438 (14, 14, $M^{+\cdot}$); 398, 396 (12, 12, $[M - CH_2CO]^+$); 366, 364 (12, 12 $[M - CH_2CO - MeOH]^+$); 359 (28, $[M - Br]^+$); 317 (23, $[M - CH_2CO - Br]^+$); 225 (48); 43 (100). HR-MS: 438.053 ± 0.004 ($C_{17}H_{19}{}^{79}BrN_4O_5^+$, calc. 438.054).

Data of **15**: ^{1}H-NMR ($CDCl_3$): 6.42 (*d*, $J(2,3) = 4.1$, H–C(2)); 7.21 (*d*, $J(3,2) = 4.1$, H–C(3)); 2.47 (*s*, AcO–N(5)); 5.48 (*dd*, $J(5a,5b) = 8.1$, $J(5a,9a) = 5.1$, H–C(5a)); 4.58 (*d*, $J(5b,5a) = 8.1$, H–C(5b)); 2.59 (*s*, AcO–N(6)); 2.85 (*s*, Me–N(8)); 2.82 (*s*, MeO); 3.73 (*d*, $J_{gem} = 15.4$, H_α–C(9)); 2.40 (*dd*, $J_{gem} = 15.4$, $J(9\beta,9a) = 5.3$, H_β–C(9)); 4.62 (*t*, $J(9a,5a) = 5.1$, $J(9a,9\beta) = 5.3$, H–C(9a)). ^{13}C-NMR ($CDCl_3$): 106.99 (*s*, C(1)); 116.26 (*d*, C(2)); 119.14 (*d*, C(3)); 125.77 (*s*, C(3a)); 155.93 (*s*, C(4)); 23.76 (*q*), 170.34 (*s*, AcO–N(5)); 59.69 (*d*, C(5a)); 61.47 (*d*, C(5b)); 26.58 (*q*), 172.40 (*s*, AcO–N(6)); 152.85 (*s*, C(7)); 24.92 (*s*, Me–N(8)); 93.26 (*s*, C(8a)); 49.66 (*s*, MeO); 41.04 (*t*, C(9)); 55.91 (*d*, C(9a)).

Data of **16**: ^{1}H-NMR ($CDCl_3$): 6.43 (*d*, $J(2,3) = 4.1$, H–C(2)); 7.20 (*d*, $J(3,2) = 4.1$, H–C(3)); 2.43 (*s*, AcO–N(5)); 5.46 (*dd*, $J(5a,9a) = 5.1$, $J(5a,5b) = 8.1$, H–C(5a)); 4.51 (*d*, $J(5b,5a) = 8.1$, H–C(5b)); 2.57 (*s*, AcO–N(6)); 2.85 (*s*, Me–N(8)); 2.92 (*s*, MeO); 3.78 (*d*, $J_{gem} = 15.4$, H_α–C(9)); 2.39 (*dd*, $J_{gem} = 15.4$, $J(9\beta,9a) = 5.3$, H_β–C(9)); 4.65 (*t*, $J(9a,5a) = 5.1$, $J(9a,9\beta) = 5.3$, H–C(9a)). ^{13}C-NMR ($CDCl_3$): 106.99 (*s*, C(1)); 116.35 (*d*, C(2)); 119.18 (*d*, C(3)); 125.88 (*s*, C(3a)); 157.88 (*s*, C(4)); 23.73 (*q*), 170.90 (*s*, AcO–N(5)); 59.62 (*d*, C(5a)); 62.31 (*d*, C(5b)); 26.54 (*q*), 172.25 (*s*, AcO–N(6)); 156.08 (*s*, C(7)); 24.82 (Me–N(8)); 89.44 (*s*, C(8a)); 53.94 (*q*, MeO); 41.26 (*t*, C(9)); 56.29 (*d*, C(9a)).

9. *5,6,8a-*O*-Triacetyl-5b,8a-diepiagelastatin A* (**17**). A soln. of **13** (10 mg) in 1 ml of Ac_2O containing catalytic DMAP was heated at 50° overnight and then evaporated. The residue was subjected to prep. TLC (CH_2Cl_2/acetone 85:15, collection of R_f 0.7 band): **8/17** 1:3 (9 mg, 90%). **17**: ^{1}H-NMR ($CDCl_3$): 6.36 (*d*, $J(2,3) = 4.2$, H–C(2)); 7.06 (*d*, $J(3,2) = 4.2$, H–C(3)); 2.38 (*s*, AcO–N(5)); 6.00 (*dd*, $J(5a,9a) = 5.1$, $J(5a,5b) = 8.7$, H–C(5a)); 5.15 (*d*, $J(5b,5a) = 8.7$, H–C(5b)); 2.50 (*s*, AcO–N(6)); 2.56 (*s*, Me–N(8)); 2.03 (*s*, AcO–C(8a)); 2.59 (*dd*, $J_{gem} = 15.6$, $J(9\beta,9a) = 6.0$, H_β–C(9)); 3.86 (*d*, $J_{gem} = 15.6$, H_α–C(9)); 4.79 (*dd*, $J(9a,9\beta) = 6.0$, $J(9a,5a) = 5.1$, H–C(9a)). ^{13}C-NMR ($CDCl_3$): 104.57 (*s*, C(1)); 116.01 (*d*, C(2)); 117.95 (*d*, C(3)); 125.85 (*s*, C(3a)); 156.37 (*s*, C(4)); 23.10 (*q*), 170.45 (*s*, AcO–N(5)); 53.64 (*d*, C(5a)); 59.19 (*d*, C(5b)); 26.18 (*q*), 171.36 (*s*, AcO–N(6)); 153.26 (*s*, C(7)); 24.86 (Me–N(8)); 94.26 (*s*, C(8a)); 21.00 (*q*), 169.12 (*s*, AcO–C(8a)); 35.66 (*t*, C(9)); 55.26 (*d*, C(9a)).

10. *5b,8a-Diepiagelastatin A* (**18**). A soln. of **12** (10 mg) in acidic H_2O/acetone 1:2 was stirred at r.t. during 15 min and then subjected to prep. TLC (CH_2Cl_2/acetone 85:15): **1/18** 2:1 (R_f 0.22; 6 mg, 60%), besides unreacted **12** (R_f 0.40; 2.5 mg, 25%). **18**: ^{1}H-NMR (CD_3OD): 6.33 (*d*, $J(2,3) = 4.2$, H–C(2)); 6.90 (*d*, $J(3,2) = 4.2$, H–C(3)); 4.43 (*t*, $J(5a,9a) = J(5a,5b) = 5.4$, H–C(5a)); 4.02 (*d*, $J(5b,5a) = 5.4$, H–C(5b)); 2.73 (*s*, Me–N(8)); 2.53 (*dd*, $J_{gem} = 13.2$, $J(9\beta,9a) = 6.9$, H_β–C(9)); 2.26 (*dd*, $J_{gem} = 13.2$, $J(9\alpha,9a) = 10.5$, H_α–C(9)); 5.00 (*ddd*, $J(9a,9\beta) = 6.9$, $J(9a,9\alpha) = 10.5$, $J(9a,5a) = 5.4$, H–C(9a)). ^{13}C-NMR (CD_3OD): 108.63 (*s*, C(1)); 115.64 (*d*, C(2)); 117.68 (*d*, C(3)); 126.27 (*s*, C(3a)); 162.92 (*s*, C(4)); 59.85 (*d*, C(5a)); 66.83 (*d*, C(5b)); 163.29 (*s*, C(7)); 26.13 (Me–N(8)); 97.20 (*s*, C(8a)); 44.20 (*t*, C(9)); 56.98 (*d*, C(9a)).

11. *8a-*O*-Methyl-5b,8a-diepiagelastatin A* (**19**). A mixture of **12** (10 mg), MeOH, and *Amberlyst 15* was heated at reflux overnight; **4/19** *ca.* 1:1. Yield 80%. HPLC (*Si-60*, CH_2Cl_2/EtOH 9:1, 3 ml/min): t_R 10 and 8.2 min, resp. **19**: ^{1}H-NMR (CD_3OD): 6.33 (*d*, $J(2,3) = 4.1$, H–C(2)); 6.90 (*d*, $J(3,2) = 4.1$, H–C(3)); 4.42 (br. *t*, $J(5a,5b) = 5.6$, $J(5a,9a) = 5.1$, $J(5a,9\beta)$ small, H–C(5a)); 4.23 (*d*, $J(5b,5a) = 5.4$, $J(5b,9\beta)$ small, H–C(5b)); 2.68 (*s*, Me–N(8)); 3.13 (*s*, MeO); 2.53 (*dd*, $J_{gem} = 13.5$, $J(9\beta,9a) = 6.9$, $J(9\beta,5a) = J(9\beta,5b)$ small, H_β–C(9)); 2.32 (*dd*, $J_{gem} = 13.5$, $J(9\alpha,9a) = 10.5$, H_α–C(9)); 4.95 (*ddd*, $J(9a,9\beta) = 6.9$, $J(9a,9\alpha) = 10.5$, $J(9a,5a) = 5.1$, H–C(9a)). ^{13}C-NMR (CD_3OD): 108.56 (*s*, C(1)); 115.69 (*d*, C(2)); 117.71 (*d*, C(3)); 126.32 (*s*, C(3a)); 162.95 (*s*, C(4)); 60.02 (*d*, C(5a)); 60.77 (*d*, C(5b)); 163.83 (*s*, C(7)); 26.38 (Me–N(8)); 101.52 (*s*, C(8a)); 51.31 (*q*, MeO); 43.66 (*t*, C(9)); 56.55 (*d*, C(9a)).

12. *5,6,8a-*O*-Trimethyl-5b,8a-diepiagelastatin A* (**20**). A mixture of **11** (10 mg), MeOH, and catalytic $(COOH)_2$ was stirred at r.t. during 1 h and then evaporated: **2/20** 1:1. Yield 95%. TLC (SiO_2, acetone): R_f 0.81 and 0.50, resp. **20**: ^{1}H-NMR (CD_3OD): 6.26 (*d*, $J(2,3) = 4.2$, H–C(2)); 6.75 (*d*, $J(3,2) = 4.2$, H–C(3)); 3.18 (*s*, Me–N(5)); 4.45 (*dd*, $J(5a,9a) = 6.0$, $J(5a,5b) = 7.5$, H–C(5a)); 4.20 (*d*, $J(5b,5a) = 7.5$, H–C(5b)); 2.74 (*s*, Me–N(6)); 2.55 (*s*, Me–N(8)); 3.03 (*s*, MeO); 3.84 (*d*, $J_{gem} = 15.6$, $J(9\alpha,9a)$ small, H_α–C(9)); 2.30 (*dd*, $J_{gem} = 15.6$, $J(9\beta,9a) = 4.8$, H_β–C(9)); 4.86 (br. *t*, $J(9a,5a) = 6.0$, $J(9a,9\beta) = 4.8$, $J(9a,9\alpha)$ small, H–C(9a)). ^{13}C-NMR (CD_3OD): 104.25 (*s*, C(1)); 115.58 (*d*, C(2)); 115.16 (*d*, C(3)); 128.08 (*s*, C(3a)); 160.71 (*s*, C(4)); 33.97 (*q*, Me–N(5)); 57.94 (*d*, C(5a)); 63.34 (*d*, C(5b)); 31.32 (*q*, Me–N(6)); 160.82 (*s*, C(7)); 24.95 (*q*, Me–N(8)); 98.16 (*s*, C(8a)); 50.44 (*q*, MeO); 37.46

(*t*, C(9)); 64.92 (*d*, C(9a)). MS: 384, 382 (8, 8, M^+); 303 (62, $[M - Br]^+$); 271 (15), 228 (40); 155 (41); 149 (42); 125 (100). HR-MS: 382.059 ± 0.006 ($C_{15}H_{19}{}^{79}BrN_4O_3^+$, calc. 382.064).

13. *6-(Methoxymethyl)-8a-*O*-methylagelastatin* (= *(5a*R*,5b*S*,8a*S*,9a*R*)-1-Bromo-5,5a,5b,6,8,8a,9,9a-octahydro-8a-methoxy-6-(methoxymethyl)-8-methylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **21**). A mixture of **4** (*ca.* 50 mg) and 10 mol-equiv. of $CH_2(OMe)_2$ in dry $CHCl_3$ (5 ml) containing 3-Å molecular sieves and *Amberlyst 15* was stirred at r.t. for 3 h and then subjected to prep. TLC (CH_2Cl_2/acetone 6:4): **21** (R_f 0.25; 30 mg, 60%). ^{1}H-NMR (CD_3OD): 6.33 (*d*, J(2,3) = 4.2, H–C(2)); 6.92 (*d*, J(3,2) = 4.2, H–C(3)); 4.33 (*d*, J(5a,9a) = 5.4, H–C(5a)); 4.24 (*s*, H–C(5b)); 3.33 (*s*), 4.65, 4.85 (*AB*, J = 10.8, $MeOCH_2$–N(6)); 2.84 (*s*, Me–N(8)); 3.16 (*s*, MeO); 2.17 (*t*, J_{gem} = 13.2, J(9α,9a) = 12.0, $H_α$–C(9)); 2.69 (*dd*, J_{gem} = 13.2, J(9β,9a) = 6.6, $H_β$–C(9)); 4.59 (*m*, J(9a,9β) = 6.6, J(9a,9α) = 12.0, J(9a,5a) = 5.4, H–C(9a)). ^{13}C-NMR (CD_3OD): 108.88 (*s*, C(1)); 115.46 (*d*, C(2)); 117.71 (*d*, C(3)); 125.67 (*s*, C(3a)); 162.59 (*s*, C(4)); 61.26 (*d*, C(5a)); 65.32 (*d*, C(5b)); 58.02 (*q*); 77.17 (*t*, $MeOCH_2$–N(6)); 161.66 (*s*, C(7)); 26.66 (*q*, Me–N(8)); 99.53 (*s*, C(8a)); 52.26 (*q*, MeO); 40.69 (*t*, C(9)); 55.27 (*d*, C(9a)).

14. *7-Bromo-1,7b,8,8a,8b,8c-hexahydro-8a-hydroxy-1-methyl-2*H,*3*H,*4*H-*1,2a,3a,7a-tetraazapentaleno[1,6-kl]-s-indacene-2,4-dione* (**22**). A mixture of **21** (30 mg) H_2O, and *Amberlyst 15* was heated under reflux overnight and then subjected to prep. TLC (CH_2Cl_2/EtOH 85:15): **22** (R_f 0.80; 6 mg, 20%) and **1**/**18** 4:1 (R_f 0.30; 12 mg). **22**: ^{1}H-NMR (CD_3OD): 6.33 (*d*, J(2,3) = 4.2, H–C(2)); 6.87 (*d*, J(3,2) = 4.2, H–C(3)); 4.87 (br. *t*, J(5a,5b) = 6.6, J(5a,9a) = 6.0, J(5a,9α) small, J(5a,9β) = 0.8, H–C(5a)); 4.28 (*dd*, J(5b,5a) = 6.6, J(5b,9β) = 1.0, J(5b,9α) small, H–C(5b)); 5.48, 4.25 (2*d*, J_{gem} = 10.2, N(5)–CH_2–N(6)); 2.72 (*s*, Me–N(8)); 2.73 (*ddt*, J_{gem} = 13.8, J(9β,9a) = 7.8, J(9β,5b) = 1.0, J(9β,5a) = 0.8, $H_β$–C(9)); 1.85 (br. *dd*, J_{gem} = 13.8, J(9α,9a) = 11.2, J(9α,5b) = J(9α,5a) small, $H_α$–C(9)); 5.14 (*ddd*, J(9a,9β) = 7.8, J(9a,9α) = 11.2, J(9a,5a) = 6.0, J(9a,3) small, H–C(9a)). ^{13}C-NMR (CD_3OD): 109.14 (*s*, C(1)); 115.67 (*d*, C(2)); 117.80 (*d*, C(3)); 125.56 (*s*, C(3a)); 165.36 (*s*, C(4)); 65.35 (*d*, C(5a)); 74.65 (*d*, C(5b)); 63.75 (*t*, N(5)–CH_2–N(6)); 157.54 (*s*, C(7)); 27.56 (*q*, Me–N(8)); 98.73 (*s*, C(8a)); 46.47 (*t*, C(9)); 55.68 (*d*, C(9a)). MS: 354, 352 (70, 71, M^+); 273 (9), 199 (29); 149 (27); 126 (32). HR-MS: 352.018 ± 0.006 ($C_{13}H_{13}{}^{79}BrN_4O_3^+$, calc. 352.018).

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