169. Conformational Preferences and Absolute Configuration of Agelastatin A, a Cytotoxic Alkaloid of the Axinellid Sponge *Agelas dendromorpha* from the Coral Sea, *via* Combined Molecular Modelling, NMR, and Exciton Splitting for Diamide and Hydroxyamide Derivatives

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Dedicated to the memory of *Mario Pietra*

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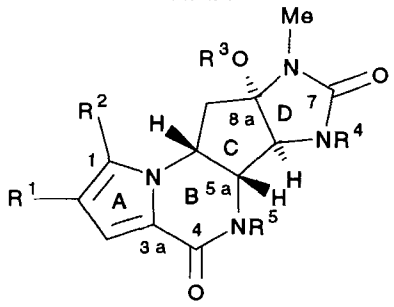
The absolute configuration of agelastatin A (**1**), the major, strongly cytotoxic alkaloid of the axinellid sponge *Agelas dendromorpha* from the Coral Sea, is proposed here to be (5a*S*,5b*S*,8a*S*,9a*R*), as deduced from combined molecular-mechanics calculations and a novel application of exciton splitting to the bis[4-(dimethylamino)benzoyl] compounds (–)-**9** and (–)-**13**, derivatives of a diamide and a hydroxyamide, respectively. The position of the conformational equilibrium of A **1** could be finely tuned by slight molecular changes. The minor analogue, agelastatin B (**3**), was isolated as the trimethyl derivative (–)-**4**.

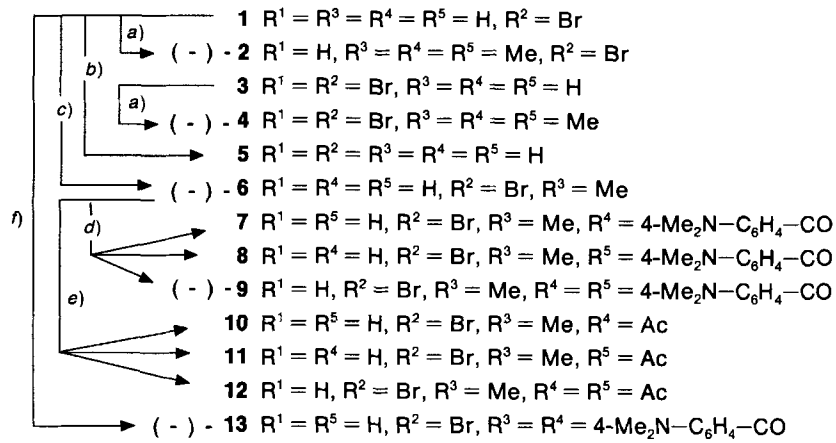
1. Introduction. – Recently, we reported on the isolation of agelastatin A (**1**) from the axinellid sponge *Agelas dendromorpha* from the Coral Sea [1]. The alkaloid was characterized through its trimethyl derivative (–)-**2** and was shown to have an unusual, new C_{11} framework which entails the compound to be a member of the oroidin family [2] of alkaloids, though the biosynthesis involves a new cyclization mode [1]. The structural novelty of agelastatin A (**1**) and its significant cytotoxicity towards tumoral cells prompted us to determine its absolute configuration and conformational behaviour as a prerequisite to understand the recognition phenomena into which it is involved. This is reported here, along with the characterisation of the dibrominated analogue agelastatin B (**3**), isolated from the same source, as its trimethyl derivative (–)-**4**.

2. Results and Discussion. – 2.1. *Agelastatin B* (**3**). The 1-monobrominated agelastatin A (**1**) is accompanied by agelastatin B (**3**), for which the structure of the 1,2-dibromo derivative was tentatively assigned from ^{1}H-NMR spectra of a inseparable mixture of **1** and **3** [1]. However, exhaustive methylation of the mixture **1**/**3** now facilitated the isolation of the trimethyl derivative (–)-**4** of **3** in pure form (*Scheme 1*). The structure of (–)-**4** rests on HR-MS and NMR spectra (*Table 1*, *Exper. Part*, and [1]) which displayed significant differences with respect to the trimethyl derivative (–)-**2** of **1** (^{1}H-NMR: no H–C(2), H–C(3) signal, assigned from deshielding by C(4)=O, simplified to *s*; ^{13}C-NMR: C(2) signal simplified to *s* and shifted to higher field; all other signals of (–)-**4** and (–)-**2** practically superimposable).

2.2. *Degradation of Agelastatin A* (**1**). We first attempted to transform agelastatin A into an amino alcohol, as intermediate en route to derivatives for exciton-coupling techniques [3]. Efforts devised to hydrolyse ring D to give an α-amino ketone failed, however. Thus, on treatment with either Na_2O_2, *t*-BuOK, or $Ba(OH)_2$ in H_2O at room temperature, agelastatin A (**1**) was left unchanged, whereas at reflux, decomposition

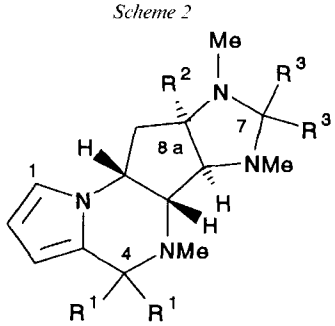
Scheme 1

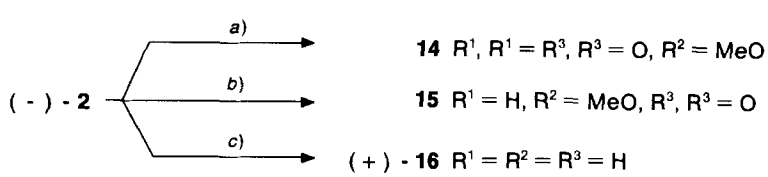




a) Dry DMSO, KOH, MeI, r.t., 30 min. *b*) $LiAlH_4$, THF, r.t., 1 h. *c*) *Amberlyst 15*, MeOH. *d*) 1. $NaN(SiMe_3)_2$, pyridine, r.t.; 2. 4-Me_2N–C_6H_4–COCl. *e*) Ac_2O, 4-(dimethylamino)pyridine, 90°, 12 h. *f*) $NaN(SiMe_3)_2$, 4-Me_2N–C_6H_4–CO–Im.

Scheme 2





a) NaH. *b*) $LiAlH_4$, THF, 0°, 1 h. *c*) $LiAlH_4$, THF, reflux, 1 h.

Table 1. *^{13}C-NMR Data for 5,6,8a-O-Trimethylagelastatin B* ((−)-**4**) *and Various Derivatives of Agelastatin A* (**1**)

	(−)-**4**[a])	(−)-**6**[a])	(−)-**9**[b])	**12**[b])	(−)-**13**[b])	**14**[a])	**15**[a])	(+)-**16**[a])
C(1)	109.55 (*s*)	108.89 (*s*)	105.74 (*s*)	106.98 (*s*)	105.46 (*s*)	126.19 (*d*)	120.81 (*d*)	120.80 (*d*)
C(2)	103.68 (*s*)	115.42 (*d*)	113.94 (*d*)	116.25 (*d*)	113.08 (*d*)	112.89 (*d*)	111.04 (*d*)	110.79 (*d*)
C(3)	118.65 (*d*)	117.65 (*d*)	117.21 (*d*)	119.12 (*d*)	115.35 (*d*)	116.99 (*d*)	105.05 (*d*)	104.84 (*d*)
C(3a)	126.33 (*s*)	125.63 (*s*)	123.23 (*s*)	125.79 (*s*)	122.93 (*s*)	125.69 (*s*)	127.85 (*s*)	127.77 (*s*)
C(4)	160.93 (*s*)	162.50 (*s*)	157.77 (*s*)	155.93 (*s*)	158.31 (*s*)	161.93 (*s*)	55.20 (*t*)	55.85 (*t*)
R–N(5)	33.19 (*q*)	–	172.14 (*s*)[c])	170.32 (*s*)[d])	–	36.13 (*q*)	44.64 (*q*)	44.74 (*q*)
C(5a)	66.39 (*d*)	63.57 (*d*)	63.07 (*d*)	59.69 (*d*)	59.36 (*d*)	71.64 (*d*)	71.00 (*d*)	73.63 (*d*)
C(5b)	67.17 (*d*)	62.69 (*d*)	59.92 (*d*)	61.48 (*d*)	66.84 (*d*)	69.89 (*d*)	66.19 (*d*)	74.00 (*d*)
R–N(6)	30.69 (*q*)	–	168.43 (*s*)[c])	172.37 (*s*)[d])	164.84 (*s*)[e])	31.91 (*q*)	30.51 (*q*)	40.79 (*q*)
C(7)	162.14 (*s*)	163.36 (*s*)	154.15 (*s*)	152.84 (*s*)	153.97 (*s*)	162.43 (*s*)	162.52 (*s*)	82.64 (*t*)
Me–N(8)	26.65 (*q*)	26.20 (*q*)	25.04 (*q*)	24.94 (*q*)	25.55 (*q*)	26.64 (*q*)	26.61 (*q*)	40.64 (*q*)
RO–C(8a)	52.17 (*q*)	52.22 (*q*)	50.32 (*q*)	49.67 (*q*)	168.92 (*s*)[e])	–[f])	51.91 (*q*)	–
C(8a)	98.30 (*s*)	101.67 (*s*)	94.87 (*s*)	93.27 (*s*)	93.49 (*s*)	99.28 (*s*)	98.96 (*s*)	78.60 (*d*)
C(9)	41.48 (*t*)	40.71 (*t*)	40.48 (*t*)	41.05 (*t*)	38.26 (*t*)	42.76 (*t*)	43.18 (*t*)	41.06 (*t*)
C(9a)	56.07 (*d*)	55.30 (*d*)	53.15 (*d*)	55.93 (*d*)	51.55 (*d*)	57.40 (*d*)	56.90 (*d*)	58.84 (*d*)

[a]) In CD_3OD.

[b]) In $CDCl_3$.

[c]) 121.98 and 119.90 (2 *s*, C(1′)); 132.33 and 132.51 (2 *d*, C(2′)); 109.96 and 110.66 (2 *d*, C(3′)); 153.44 and 153.87 (2 *s*, C(4′)); 40.03 (4 *q*, Me_2N).

[d]) 23.79 and 26.62 (2 *q*, *Me*CO).

[e]) 128.77 and 119.64 (2 *s*, C(1′)); 131.86 (2 *d*, C(2′)); 110.63 and 109.89 (2 *d*, C(3′)); 153.81 and 153.02 (2 *s*, C(4′)); 39.97 (4 *q*, Me_2N).

[f]) Submerged by residual solvent signals.

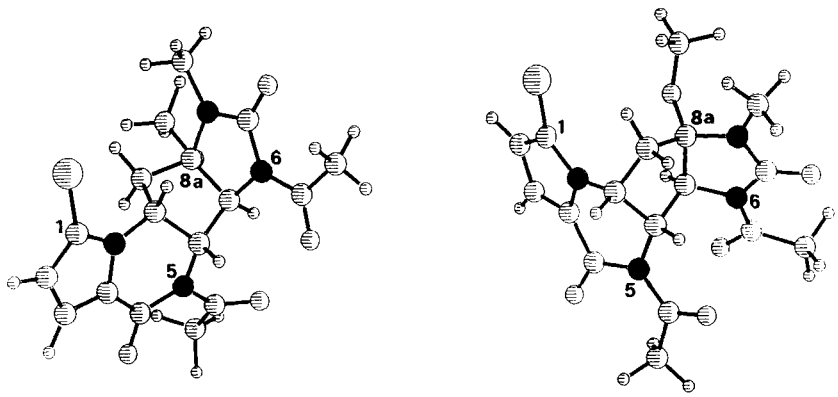
yielded only 5-bromopyrrole-2-carboxylic acid. On treatment with the strongly acidic resin *Amberlyst 15*, **1** was left unchanged in H_2O, whereas in MeOH, 5-*O*-methylation gave (−)-**6** [4a]. Since imidazolidines are known to undergo facile hydrolysis [5], we hoped that hydrolytic ring-D opening could be achieved through a CH_2(7) derivative. Treatment of **1** with $LiAlH_4$, however, only furnished **5** by reductive debromination, formally *via* conjugate addition of hydride [4b].

Before finding the way to the absolute configuration of agelastatin A (**1**) *via* compounds **7** to (−)-**13**, treatment of the trimethyl derivative (−)-**2** with NaH was found to induce reductive debromination, giving **14**, whereas $LiAlH_4$ induced both debromination and reduction at C(4) affording **15** in THF at 0° or, at reflux, reductive replacement of O at C(4), C(7), and C(8a), furnishing (+)-**16** (*Scheme 2*). The reactivity at C(8a) may reflect the amino-acetal nature of this centre [6b] but, contrary to expectations [5], imidazolidine (+)-**16** failed to eliminate formaldehyde under either basic (*t*-BuOK in THF at room temperature) or acidic (*Amberlyst 15*/MeOH or HCl/aq. MeOH) conditions. This knowledge should turn out to be useful in programs of structure-bioactivity and total synthesis of agelastatin A and analogues.

2.3. *Conformational Analysis and Absolute Configuration from Exciton Coupling Applied to Diamide or Hydroxyamide Systems.* We then focused our attention to the *N*-monosubstituted amide groups CO(4)–N(5) and CO(7)–N(6) of agelastatin A (**1**). Should the electric transition moments of suitable chromophores R^4 and R^5 have the same directions as those for the C(5b)–N(6) and C(5a)–N(5) bonds, the exciton-coupling technology for diols [7a], polyols [7b], amino alcohols [7c], and diamines [8] could be extended to this diamide system.

From examination of mechanical models, however, the agelastatins appeared to be quite flexible at ring C, a conclusion in agreement with the observed change in the

H-NMR coupling pattern of H–C(5b), which appeared as a *s* for both agelastatin A (**1**) and its trimethyl derivative (–)-**2**, or as a *d* for both 1-debromoagelastatin A (**5**) and the methoxy analogue **14**, with smaller coupling for **5** than **14** (*Exper. Part*). Moreover, molecular-mechanics (MM) calculations for **1** suggested the existence of two low-energy, rapidly equilibrating conformers originating from flipping at ring C by rotation around the C(5b)–C(5a)–C(9a)–C(9) dihedral angle. In the major conformer, the H–N(5) and H–N(6) bonds are orthogonal to one another, from which the term 'crossed' conformer (c-**1**) arises (see c-**12** in the *Fig.*, with H at both N(5) and N(6) instead of AcO and OH at



c-12 p-12

Figure. *Crossed-type* (c) *and parallel-typ* (p) *conformers of diacetyl derivative* **12** *as output of MM calculations.* All other cases of c- and p-conformers can be grasped from the text in relation to c- and p-**12**, replacing, as appropriate, the substituents at C(1), N(5), N(6), and C(8a).

C(8a) instead of MeO). In the minor conformer, the H–N(5) and H–N(6) bonds are in plane, from which the term 'parallel' conformer (p-**1**) arises (see p-**12** in the *Fig.*). In addition, the dihedral angle H(5b)–C(5b)–C(5a)–H(5a) was calculated to be *ca.* 90 or 180° for c-**1** and p-**1**, respectively, in agreement with the observed *J* values (*Table 2*).

Similar calculations for the permethylated derivative (–)-**2** pointed to analogous conformers, with the 'crossed' conformer c-**2** being slightly less favoured than in the case of **1** because of steric repulsions between H–C(5b) and Me–N(5). These calculations indicated also that the 'crossed' conformer c-**5** for 1-debromoagelastatin A is even less favoured (*Table 2*), due to absence of interference of the Br-atom with H_2–C(9) in the 'parallel' conformer. All this fits with the observed coupling constants for both (–)-**2** and **5** (*Table 2*).

As indicated by MM calculations, both factors also influence the conformational equilibrium of permethylated 1-debromoagelastatin A **14**, revealing the dominance of the 'parallel' over the 'crossed' conformer, in agreement with the observed averaged *J* couplings (*Table 2*).

It is important to note that chromophores R^5 and R^4 at N(5) and N(6) give rise, in each of the above couples, to the same sign of chirality [3] in both conformers, which is an essential premise in view of applying exciton-coupling rules [3]. Thus, we examined the

Table 2. *Two-States Fast Conformational Equilibrium for the 'Parallel' and 'Crossed' Conformers of Agelastatin A* (**1**) *and Its Derivatives* **2**, **5**, **12**, *and* **14**

	ΔE[a])	x_c[b]), x_p[c])	$J_{calcd.}$				$J_{obsd.}$[d])	
			$J_{(9a,9\alpha)}$	$J_{(5a,5b)}$	$\bar{J}_{(9a,9\alpha)}$[b])	$\bar{J}_{(5a,5b)}$[e])	$J_{(9a,9\alpha)}$	$J_{(5a,5b)}$
c-**1**	2.5	0.99	11.2	0.7	11.1	0.8	12.3	≤ 0.5
p-**1**		0.01	1.8	8.1				
c-**2**	1.5	0.93	11.5	0.9	11	1.4	11.9	≤ 0.5
p-**2**		0.07	2.1	8.6				
c-**5**	0.8	0.8	11.1	0.7	9.2	2.1	10.2	1.8
p-**5**		0.2	1.7	8				
c-**9**	−0.7	0.24	11.5	0.7	4.1	8.4	6.6	4.2
p-**9**		0.76	1.8	11				
c-**12**	−1.9	0.03	11.5	0.7	2.1	10	≤ 0.5	8.7
p-**12**		0.97	1.8	11				
c-**14**	−0.4	0.35	11.4	1.0	5.3	6.1	3.8	5.4
p-**14**		0.65	2.0	8.8				

[a]) ΔE = Difference of strain energies (kcal mol^{-1}) between the crossed-type (c) and parallel-type (p) conformers, evaluated from MM calculations, dielectric constant 30.

[b]) x_c = Molar fraction for the crossed-type conformer, calculated from $x_c = 1/(1 + K)$.

[c]) x_p = Molar fraction for the parallel-type conformer, calculated from $x_p = Kx_c$, where $K = \exp(-\Delta E/RT)$, T 298°K.

[d]) In CD_3OD.

[e]) Average values.

Ac group as substituents at both N(5) and N(6) (see **12**) prior to the study of di-*N*-benzoyl-substituted systems. MM Calculations suggested the dominance of the 'parallel' (p-**12**) over the 'crossed' conformer c-**12** by 0.97/0.03, a ratio supported by the observed J values (*Table 2*). Energy factorization in the MM calculations suggested that dipole-dipole interactions dominate in this case, being either attracting forces in the oppositely superimposed acetyl C=O group in the 'parallel' conformer p-**12** or repulsive forces in the facing acetyl C=O groups in the 'crossed' conformer c-**12**. We were thus ready for considering a dibenzoyl derivative. MM Calculations suggested for 5,6-bis[4-(dimethylamino)benzoyl] derivatives a situation similar to the 5,6-diacetyl derivative **12**, albeit with less marked preference for the 'parallel' over the 'crossed' conformer (p-**9**/c-**9** 76/0.24, see *Table 2* and *Fig.*).

$NaN(SiMe_3)_2$-Induced benzoylation of (−)-**6** with 4-Me_2N–C_6H_4–COCl gave the dibenzoyl derivative (−)-**9**[1]), accompanied by the monobenzoylated derivatives **7** and **8**, from which (−)-**9** was separated by TLC (*Scheme 1*). From the ^{1}H-NMR spectra of (−)-**9**, it became clear that the 'crossed' conformer c-**9** was more dominant than predicted by MM calculations. This is not surprising, since equilibrium concentrations of p- and c-**9** may depend on subtle factors introduced by (dimethylamino)phenyl rings replacing the Me groups of **12**. Nevertheless, these results suggest that conformers p- and c-**9** both have

[1]) Efforts devised to prepare the corresponding bis(4-methoxycinnamoyl) derivative failed; only mono(4-methoxycinnamoyl) derivatives were obtained.

a negative sign of chirality [3] (*Fig.*), allowing the application of exciton-coupling rules [3]. CD Spectra for (−)-**9** in EtOH gave exciton splitting with negative first and positive second *Cotton* effects, pointing to the absolute configuration (5a*R*,5b*S*,8a*S*,9a*R*)-**9**, *i.e.*, (5a*S*,5b*S*,8a*S*,9a*R*)-**1**, as represented in *Scheme 1*.

In view of absence of application of the exciton-coupling technology to diamides, we decided to confirm the above conclusions using another derivative of **1**. In this context, it was particularly helpful that we discovered that (−)-**6** could be monobenzylated at N(6) to give **7** by means of 1-[4-(dimethylamino)benzoyl]-1*H*-imidazole, easily obtained from the free acid and 1,1′-carbonylbis(1*H*-imidazole). The same selectivity was observed on benzoylation of **1**[2]), providing an easy access to (−)-**13** (*Scheme 1*). The CD spectrum of (−)-**13** indicated a positive sign of chirality with respect to the C(5b)–N(6) and C(8a)–O bonds, confirming, in the absence of conformational problems, the absolute configuration of **1** as depicted in *Scheme 1*.

We thank Mrs *S. Gadotti* and *A. Sterni* for skilled technical aid with the isolation of compounds and mass spectra, respectively. Financial support by *MURST* (Progetti 40%) and *CNR*, Roma, is also acknowledged. This project was started as isolation of new metabolites [1] with the collaborative program *ORSTOM-CNRS* on Marine Substances of Biological Interest.

Experimental Part

1. *General. Amberlyst 15* was purchased from *Rohm & Haas Co.* Data for derivatives **7**, **8**, **10**, and **11** – irrelevant to the present purpose – are not reported here but are available from the authors on request. Molecular-mechanics calculations: MMX, PCMOD 4.0, *Serena Software*, Bloomington, Indiana. All evaporations were carried out at reduced pressure below 40°. Flash chromatography (FC): *Merck Si-60* (15–25 μm) or *Merck RP-18 LiChroprep* (40–65 μm). TLC: *Merck* silica gel *60 PF_{254}* plates. HPLC: *Merck LiChrosorb Si-60* (7 μm), 25 × 1 cm column; solvent flow 5 ml min^{-1}; UV monitoring at λ 254 nm. UV: *Perkin-Elmer-Lambda-3* spectrophotometer; λ_{max} in nm, ε in mol^{-1} l cm^{-1}. Polarimetric data: *JASCCO-DIP-181* polarimeter; $[\alpha]_D$ in dm^{-1} deg ml g^{-1}. CD: *Jasco-J-710* spectropolarimeter; $\Delta\varepsilon$ (λ). IR: *Perkin-Elmer-337* spectrometer; ν_{max} in cm^{-1}. NMR: δ in ppm rel. to internal $SiMe_4$ (= 0 ppm), J in Hz ('small' stands for J < 0.5 Hz); *Varian-XL-300* spectrometer; ^1H at 299.94 MHz, ^{13}C at 75.4 MHz; assignments confirmed by $^1H,^{13}C$ COSY [10]; differential NOE (obtained with 6 s preirradiation) are reported as 'irradiated proton(s)→ % NOE proton(s)'. EI-MS (*m/z* (%)): *Kratos-MS80* spectrometer with home-built data system.

2. *5,6,8a-*O*-Trimethylagelastatin B* (= *(−)-(5a*R*,5b*S*,8a*S*,9a*R*)-1,2-Dibromo-5,5a,5b,6,8,8a,9,9a-octahydro-8a-methoxy-5,6,8-trimethylimidazo[4′,5′:4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; (−)-**4**). To a soln. of the original [1] mixture of agelastatin A (**1**) and agelastatin B (**3**; 35 mg) in dry DMSO (0.75 ml) was added finely crushed KOH, and the suspension was stirred for 10 min. Then, excess MeI was added, stirring continued for 0.5 h, H_2O added, and the mixture neutralized with NaH_2PO_4 and subjected to reversed-phase FC (H_2O to eliminate DMSO, then MeOH), to give the methylated agelastatins. The latter were subjected to HPLC (CH_2Cl_2/EtOH 96.5:3.5): (−)-**4** [1] (6 mg; t_R 8.8 min) and (−)-**2** (25 mg; t_R 10.0 min). (−)-**4**: $[\alpha]_D^{20} = -84.4$ (EtOH, $c = 0.49$). UV (EtOH): 284 (8700), 239 (8500), 204 (13200). IR (KBr): 1710, 1650, 1440. ^{1}H-NMR (CD_3OD): 6.94 (*s*, H–C(3)); 3.18 (*s*, Me–N(5)); 4.26 (br. *d*, J(5a,9a) = 6.0, J(5a,5b) small, H–C(5a)); 4.30 (br. *s*, J(5b,9β), J(5b,9α) and J(5b,5a) small, H–C(5b)); 2.97 (*s*, Me–N(6)); 2.81 (*s*, Me–N(8)); 3.13 (*s*, MeO–C(8a)); 2.17 (*B* of *ABX*, further long-range-coupled, $J(A,B)$ = 13.0, $J(B,X)$ = 12.0, J(9α,5b) small, H_α–C(9)); 2.70 (*A* of *ABX*, further long-range-coupled, $J(A,B)$ = 13.0, $J(A,X)$ = 6.0, J(9β,5b) small, H_β–C(9)); 4.68 (*X* of *ABX*, further coupled, J(9a,9β) = J(9a,5a) = 6.0, J(9a,9α) = 12.0, H–C(9a)). MS: 464, 462, 460 (6, 12, 6, $M^{+\cdot}$); 383, 381 (39, 39, $[M - Br]^+$); 351, 349 (19, 19, $[M - Br - MeO]^+$). HR-MS: 459.973 ± 0.003 ($[C_{15}H_{18}{}^{79}Br_2N_4O_3]^+$, calc. 459.974); 461.972 ± 0.003 ($[C_{15}H_{18}{}^{79}Br^{81}BrN_4O_3]^+$, calc. 461.972); 463.971 ± 0.004 ($[C_{15}H_{18}{}^{81}Br_2N_4O_3]^+$, calc. 463.970).

3. *1-Debromoagelastatin A* (= *(5a*S*,5b*S*,8a*S*,9a*R*)-5,5a,5b,6,8,8a,9,9a-Octahydro-8a-hydroxy-8-methylimidazo[4′,5′:4,5]cyclopenta[1,2-*c*]pyrrolo[1,2-*a*]pyrazine-4,7-dione*; **5**). Under N_2, **1** (10 mg) and excess $LiAlH_4$ were

[2]) Selectivity at N(6) was also found in the reaction of 4-methoxycinnamic anhydride [9] with (−)-**6**.

heated under reflux in dry THF for 1 h. AcOEt and then H_2O were added, and the mixture was subjected to prep. TLC (AcOEt/EtOH 85:15): **5** (6 mg, 60%; R_f 0.2). ^{1}H-NMR (CD_3OD): 7.03 (*dd*, $J(1,2) = 2.4$, $J(1,3) = 1.5$, H–C(1)); 6.23 (*dd*, $J(2,1) = 2.4$, $J(2,3) = 3.9$, H–C(2)); 6.89 (*dd*, $J(3,1) = 1.5$, $J(3,2) = 3.9$, H–C(3)); 4.01 (*dd*, $J(5a,9a) = 5.2$, $J(5a,5b) = 1.8$, H–C(5a)); 3.81 (*d*, $J(5b,5a) = 1.8$, H–C(5b)); 2.80 (*s*, Me–N(8)); 2.29 (*B* of *ABX*, $J(A,B) = 13.5$, $J(B,X) = 10.2$, H_α–C(9)); 2.62 (*A* of *ABX*, $J(A,B) = 13.5$, $J(A,X) = 6.3$, H_β–C(9)); 4.66 (*X* of *ABX*, further coupled, $J(9a,9\beta) = 6.3$, $J(9a,9\alpha) = 10.2$, $J(9a,5a) = 5.2$, H–C(9a)).

4. *8a-*O*-Methylagelastatin A* (= *(−)-(5aS,5bS,8aS,9aR)-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-4,7-dione*; (−)-**6**). Overnight **1** (10 mg) was heated under reflux in MeOH in the presence of *Amberlyst 15*. The mixture was then filtered and evaporated to give (−)-**6** (8 mg, 80%). $[\alpha]_D^{20} = -68.1$ (EtOH, $c = 0.32$). UV (EtOH): 277 (11500), 229 (8200), 201 (11000). ^{1}H-NMR (CD_3OD): 6.33 (*d*, $J(2,3) = 3.9$, H–C(2)); 6.92 (*d*, $J(3,2) = 3.9$, H–C(3)); 4.12 (*d*, $J(5a,9a) = 5.4$, H–C(5a)); 4.09 (*s*, H–C(5b)); 2.78 (*s*, Me–N(8)); 3.18 (*s*, MeO–C(8a)); 2.13 (*B* of *ABX*, $J(A,B) = 12.9$, $J(B,X) = 12.3$, H_α–C(9)); 2.65 (*A* of *ABX*, $J(A,B) = 12.9$, $J(A,X) = 6.6$, H_β–C(9)); 4.62 (*X* of *ABX*, further coupled, $J(9a,9\beta) = 6.6$, $J(9a,9\alpha) = 12.3$, $J(9a,5a) = 5.4$, H–C(9a)). MS: 356, 354 (33, 26, $M^{+\cdot}$); 324, 322 (5, 4, $[M - MeOH]^{+\cdot}$); 275 (25, $[M - Br]^+$); 243 (24, $[M - Br - MeOH]^{+\cdot}$); 214 (68); 28 (100). HR-MS: 354.033 ± 0.004 ($[C_{13}H_{15}{}^{79}BrN_4O_3]^+$, calc. 354.033); 356.032 ● 0.003 ($[C_{13}H_{15}{}^{81}BrN_4O_3]^+$, calc. 356.031).

5. *5,6-Bis[4-(dimethylamino)benzoyl]-8a-*O*-methylagelastatin A* (= *(−)-(5aR,5bS,8aS,9aR)-1-Bromo-5,6-bis[4-(dimethylamino)benzoyl]-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-4,7-dione*; (−)-**9**). To a soln. of (−)-**6** (6 mg) in dry pyridine (1 ml) were added a few mg of $NaN(SiM_3)_2$, and the mixture was stirred for 30 min. Then, 4-Me_2N–C_6H_4–COCl was added in 2.5 molar excess, the mixture heated at 80° for 4 h, the solvent evaporated, and the residue subjected to prep. TLC (CH_2Cl_2/AcOEt 1:1): (−)-**9** (2.4 mg; R_f 0.8) and **7**/**8** (4.0 mg; R_f 0.3). (−)-**9**: $[\alpha]_D^{20} = -135.0$ (EtOH, $c = 0.13$). UV (EtOH): 340 (37300). CD (EtOH): −12.3 (355), 0.00 (340), +9.8 (326). ^{1}H-NMR (CD_3OD): 6.47 (*d*, $J(2,3) = 3.9$, H–C(2)); 7.06 (*d*, $J(3,2) = 3.9$, H–C(3)); 4.96 (*dd*, $J(5a,9a) = 5.1$, $J(5a,5b) = 4.2$, H–C(5a)); 5.00 (*d*, $J(5b,5a) = 4.2$, H–C(5b)); 2.84 (*s*, Me–N(8)); 2.86 (*s*, MeO–C(8a)); H_α–C(9) submerged at 3.06; H_β–C(9) submerged at 2.8; 5.03 (*ddd*, $J(9a,9\beta) = 5.5$, $J(9a,9\alpha) = 6.6$, $J(9a,5a) = 5.1$, H–C(9a)); 7.67, 7.69, 6.69, 6.71 (4*d*, $J = 9.0$, 8 arom. H); 3.06, 3.07 (2*s*, 2 Me_2N).

6. *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-4,7-dione*; **12**). Overnight, (−)-**6** (6 mg) and catalytic 4-(dimethylamino)pyridine in excess Ac_2O were heated at 90°. The mixture was then evaporated and subjected to prep. TLC (CH_2Cl_2/acetone 7:3): **12** (5.4 mg; R_f 0.8) and **10**/**11** (0.5 mg; R_f 0.5). **12**: ^{1}H-NMR (CD_3OD): 6.47 (*d*, $J(2,3) = 4.2$, H–C(2)); 7.18 (*d*, $J(3.2,) = 4.2$, H–C(3)); 2.82, 2.84 (2*s*, MeCO–N(5), MeCO–N(6)); 5.53 (*dd*, $J(5a,5b) = 8.7$, $J(5a,9a) = 5.1$, H–C(5a)); 4.54 (*d*, $J(5b,5a) = 8.7$, H–C(5b)); 2.84 (*s*, Me–N(8)); 2.81 (*s*, MeO–C(8a)); 3.68 (*d*, $J(9\alpha,9\beta) = 15.3$, H_α–C(9)); 2.67 (*dd*, $J(9\beta,9\alpha) = 15.3$, $J(9\beta,9a) = 5.1$, H_β–C(9)); 4.75 (*dd*, $J(9a,9\beta) = J(9a,5a) = 5.1$, H–C(9a)).

7. *6,8a-*O*-Bis[4-(dimethylamino)benzoyl]agelastatin A* (= *(−)-(5aR,5bS,8aS,9aR)-1-Bromo-6-[4-(dimethylamino)benzoyl]-4,5,5a,5b,6,7,8,8a,9,9a-decahydro-8-methyl-4,7-dioxoimidazo[4′,5′:4,5]cyclopenta[1,2-e]pyrrolo[1,2-a]pyrazin-8a-yl 4-(Dimethylamino)benzoate*; (−)-**13**). To a soln. of **1** (21 mg) in dry THF (2 ml) were added a few mg of $NaN(SiMe_3)_2$ and 3 mol-equiv. of 1-[4-(dimethylamino)benzoyl]-1*H*-imidazole. The mixture was stirred overnight and then evaporated and the residue subjected to prep. TLC (CH_2Cl_2/acetone 4:6): (−)-**13** (11.5 mg; R_f 0.8). $[\alpha]_D^{20} = -39.6$ ($CHCl_3$, $c = 0.28$). UV ($CHCl_3$): 327 (39800), 290 (16000). CD ($CDCl_3$): +18.9 (336), 0.00 (324), −16.2 (311). ^{1}H-NMR ($CDCl_3$): 6.30 (*d*, $J(2,3) = 4.2$, H–C(2)); 6.98 (*d*, $J(3,2) = 4.2$, H–C(3)); 6.08 (br. *s*, H–N(5)); 4.42 (br. *d*, $J(5a,9a) = 6.3$, H–C(5a)); 4.88 (br. *s*, H–C(5b)); 2.88 (*s*, Me–N(8)); 2.63 (*dd*, $J_{gem} = 12.7$, $J(9\alpha,9a) = 12.0$, H_α–C(9)); 3.01 (br. *dd*, $J_{gem} = 12.7$, $J(9\beta,9a) = 6.3$, H_β–C(9)); 4.63 (*ddd*, $J(9a,9\alpha) = 12.0$, $J(9a,9\beta) = J(9a,5a) = 6.3$, H–C(9a)); 7.79, 7.68, 6.61, 6.58 (4*d*, $J = 9.0$, 8 arom. H); 3.03, 3.00 (2*s*, 2 Me_2N).

8. *1-Debromo-5,6,8a-*O*-trimethylagelastatin A* (= *(5aR,5bS,8aS,9aR)-5,5a,5b,6,8,8a,9,9a-Octahydro-8a-methoxy-5,6,8-trimethylimidazo[4′,5′:4,5]cyclopenta[1,2-e]pyrrolo[1,2-a]pyrazine-4,7-dione*; **14**). Under N_2, 5,6,8a-*O*-trimethylagelastatin A ((−)-**2**; 5 mg) and excess NaH were heated at 50° in dry THF for 1 h. The mixture was then subjected to prep. TLC (CH_2Cl_2/Me_2CO 1:1): **14** (3 mg, 60%; R_f 0.2). ^{1}H-NMR (CD_3OD): 7.02 (br. *dd*, $J(1,2) = 2.7$, $J(1,3) = 1.5$, $J(1,9a)$ small, H–C(1)); 6.25 (*dd*, $J(2,3) = 3.9$, $J(2,1) = 2.7$, H–C(2)); 6.88 (*dd*, $J(3,2) = 3.9$, $J(3,1) = 1.5$, H–C(3)); 3.23 (*s*, Me–N(5)); 4.06 (*dd*, $J(5a,9a) = J(5a,5b) = 5.4$, H–C(5a)); 3.81 (br. *d*, $J(5b,5a) = 5.4$, $J(5b,9\alpha)$ small, H–C(5b)); 2.98 (*s*, Me–N(6)); 2.79 (*s*, Me–N(8)); 2.86 (*s*, MeO–C(8a)); 2.75 (*B* of *ABX*, further long-range coupled, $J(A,B) = 14.7$, $J(B,X) = 3.8$, $J(9\alpha,5b)$ small, H_α–C(9)); 2.53 (*A* of *ABX*, further

long-range coupled, $J(A,B) = 14.7$, $J(A,X) = 5.4$, H_β–C(9)); 4.66 (*X* of *ABX*, further coupled, $J(9a,9\beta) = J(9a,5a) = 5.4$, $J(9a,9\alpha) = 3.8$, $J(9a,1)$ small, H–C(9a)). NOE: 7.02→2% on 2.75 and 4% on 6.25; 6.88→1% on 6.25; 3.23→9% on 4.06; 2.98→7% on 3.81; 2.86→15% on 3.81; 2.75→4% on 7.02. MS: 304 (27, M^+), 125 (100). HR-MS: 304.154 ± 0.003 ($[C_{15}H_{20}N_4O_3]^+$, calc. 304.153).

9. *1-Debromo-4-deoxo-5,6,8a-*O*-trimethylagelastatin A* (= *(5a*R*,5b*S*,8a*S*,9a*R*)-5,5a,5b,6,8,8a,9,9a-Octahydro-8a-methoxy-5,6,8-trimethylimidazo[4',5':4,5]cyclopenta[1,2-e]pyrrolo[1,2-a]pyrazine-7-(4*H*)-one*; **15**). Under N_2, (–)-**2** (17 mg) was stirred in dry THF containing excess $LiAlH_4$ at 0° for 1 h. AcOEt and then H_2O were added, and the surnatant was subjected to prep. TLC (CH_2Cl_2/Me_2CO 1:1): **15** (11 mg, 65%; R_f 0.6). ^{1}H-NMR (CD_3OD): 6.63 (*m*, $J(1,2) = 2.7$, $J(1,3) = 1.8$, $J(1,4\beta) = 0.9$, H–C(1)); 6.04 (*dd*, $J(2,3) = 3.6$, $J(2,1) = 2.7$, H–C(2)); 5.76 (br. *m*, $J(3,2) = 3.6$, $J(3,4\beta) = J(9,1) = 1.8$, $J(3,4\alpha) = 0.6$, H–C(3)); 3.94 (br. *d*, $J_{gem} = 14.4$, $J(4\alpha,3) = 0.6$, $J(4\alpha,5a)$ small, H_α–C(4)); 3.28 (br. *d*, $J_{gem} = 14.4$, $J(4\beta,3) = 1.8$, $J(4\beta,1) = 0.9$, $J(4\beta,5a)$ small, H_β–C(4)); 2.53 (*s*, Me–N(5)); 2.81 (br. *d*, $J(5a,9a) = 5.7$, $J(5a,9\beta) = 0.5$, $J(5a,9\alpha) = 1.0$, $J(5a,5b) = J(5a,4\alpha) = J(5a,4\beta)$ small, H–C(5a)); 4.00 (br. *s*, $J(5b,9\alpha)$, $J(5b,9\beta)$ and $J(5b,5a)$ small, H–C(5b)); 2.92 (*s*, Me–N(6)); 2.79 (*s*, Me–N(8)); 3.09 (*s*, MeO–C(8a)); 2.20 (*B* of *ABX*, further coupled, $J(A,B) = 12.9$, $J(B,X) = 11.7$, $J(9\alpha,5a) = 1.0$, $J(9\alpha,5b)$ small, H_α–C(9)); 2.62 (*A* of *ABX*, further long-range coupled, $J(A,B) = 12.9$, $J(A,X) = 6.6$, $J(9\beta,5a) = 0.5$, $J(9\beta,5b)$ small, H_β–C(9)); 4.32 (*X* of *ABX*, further coupled, $J(9a,9\beta) = 6.6$, $J(9a,9\alpha) = 11.7$, $J(9a,5a) = 5.7$, H–C(9a)). NOE: 6.63→5% on 4.32; 5.76→2% on 3.94; 3.94→2% on 5.76; 3.28→1% on 2.81; 2.53→14% on 4.00 and 8% on 3.94; 2.92→5% on 4.00; 3.09→8% on 4.00; 4.32→7% on 2.81, 5% on 6.63, and 4% on 2.62. MS: 290 (27, M^+), 259 (14), 125 (100). HR-MS: 290.174 ± 0.002 ($[C_{15}H_{22}N_4O_2]^+$, calc. 290.174).

10. *1-Debromo-4,6-dideoxo-5,6,8a-*O*-trimethylagelastatin A* (= *(+)-(5a*R*,5b*R*,8a*R*,9a*R*)-4,5,5a,5b,6,7,8,8a,9,9a-Decahydro-5,6,8-trimethylimidazo[4',5':4,5]cyclopenta[1,2-e]pyrrolo[1,2-a]pyrazine*; (+)-**16**). As described in *Exper. 9* with (–)-**2** (20 mg) at 60°. Prep. TLC (CH_2Cl_2/Me_2CO 3:7) gave (+)-**16** (11 mg, 55%; R_f 0.3). $[\alpha]_D^{20} = +19.4$ (EtOH, *c* = 0.35). ^{1}H-NMR (CD_3OD): 6.62 (*m*, $J(1,2) = 2.7$, $J(1,3) = 1.8$, $J(1,4\beta) = 0.9$, H–C(1)); 6.04 (*dd*, $J(2,3) = 3.6$, $J(2,1) = 2.7$, H–C(2)); 5.75 (br. *m*, $J(3,2) = 3.6$, $J(3,1) = 1.8$, $J(3,4\beta) = 1.5$, $J(3,4\alpha) = 0.6$, H–C(3)); 3.91 (br. *d*, $J_{gem} = 14.1$, $J(4\alpha,3) = 0.6$, H_α–C(4)); 3.23 (br. *d*, $J_{gem} = 14.1$, $J(4\beta,3) = 1.5$, $J(4\beta,1) = 0.9$, H_β–C(4)); 2.43 (*s*, Me–N(5)); 2.64 (br. *d*, $J(5a,9a) = 5.6$, $J(5a,9\beta) = J(5a,9\alpha)$ small, H–C(5a)); 3.22 (*d*, $J(8a,5b) = 8.4$, H–C(5b)); 2.44 (*s*, Me–N(6)); 3.86 (*d*, $J_{gem} = 4.6$, H_α–C(7)); 2.81 (*d*, $J_{gem} = 4.6$, H_β–C(7)); 2.35 (*s*, Me–N(8)); 3.02 (*dd*, $J(5b,8a) = 8.4$, $J(8a,9\alpha) = 6.3$, H–C(8a)); 1.82 (*B* of ABX, further long-range coupled, $J(A,B) = 12.9$, $J(B,X) = 11.4$, $J(9\alpha,8a) = 6.3$, $J(9\alpha,5a)$ small, H_α–C(9)); 2.20 (*A* of *ABX*, further long-range coupled, $J(A,B) = 12.9$, $J(A,X) = 6.9$, $J(9\beta,5b) = J(9\beta,8a) = J(9\beta,5a)$ small, H_β–C(9)); 4.67 (*X* of *ABX*, further coupled, $J(9a,9\beta) = 6.9$, $J(9a,9\alpha) = 11.4$, $J(9a,5a) = 5.6$, H–C(9a)). NOE: 6.62→5% on 4.67; 5.75→2% on 3.91; 3.91→3% on 5.75; 3.23→7% on 2.64; 2.43→4% on 3.91; 2.64→6% on 3.23; 2.44→24% on 3.22 and 1% on both 2.81 and 3.86; 2.81→1% on 4.67; 2.35→8% on both 3.02 and 2.20, 3% on 2.81, and 2% on 3.86; 2.20→8% on 4.67; 4.67→4% on both 2.20 and 6.62. MS: 246 (60, M^+), 203 (7), 147 (21), 133 (49), 99 (100). HR-MS: 246.184 ± 0.002 ($[C_{14}H_{22}N_4]^+$, calc. 246.184).

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