172. From Inactive Nortopsentin D, a Novel Bis(indole) Alkaloid Isolated from the Axinellid Sponge *Dragmacidon* sp. from Deep Waters South of New Caledonia, to a Strongly Cytotoxic Derivative

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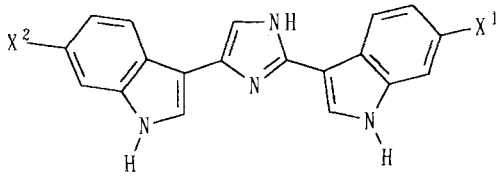
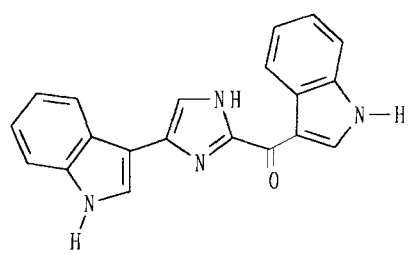
Nortopsentin D (**5**), a bis(indole) alkaloid unique for bearing a 2-amino-methylimidazole appendage at the central 1*H*-imidazol-5(4*H*)-one nucleus, was isolated in abundance, besides the putative biogenetic precursor **6** of its appendage, from the deep-water axinellid sponge *Dragmacidon* sp. Structural elucidation of **5** by NMR and MS methods heavily relied on its *N*-methyl derivatives **8**–**11**. Unusually for topsentin-type structures, natural **5** and semisynthetic methyl derivatives **8** and **10** proved inactive on KB tumoural cells, while introduction of the last three methyl groups, amazingly led to highly cytotoxic **11**.

Introduction. – Nortopsentins A–C (**1**–**3**), a new family of imidazolediylbis(indole) alkaloids, have been isolated from the halichondrid sponge *Spongosorites ruetzleri* from deep waters in the Bahamas [1]. They raised interest not only as peculiar examples of condensation between one tryptamine and one nortryptamine, but also for their inhibitory activity of both the human pathogenic yeast *Candida albicans* and P388 tumoural cells [1]. Related cytotoxic and antiviral alkaloids belong to the topsentin family [1] [2], represented here by topsentin A (**4**) [3]; they were isolated from the halichondrid sponges *S. ruetzleri* [1] [2] and *Topsentia genitrix* [3], as well as from the taxonomically and phylogenetically distant verongid sponge *Hexadella* sp. [4]. Conceivably, the topsentins derive biogenetically by head-to-head condensation of two tryptamine units.



1 $X^1 = X^2 = Br$
2 $X^1 = Br, X^2 = H$
3 $X^1 = H, X^2 = Br$



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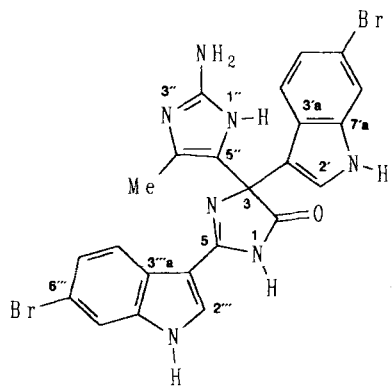
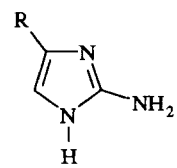
Head-to-tail condensation of two tryptamine units – resulting in a central piperazine ring – may be conceived as the biogenetic pathway towards the dragmacidins, constituting another family of bis(indole) alkaloids. They were isolated from two unrelated

sponges, *Hexadella* sp. from British Columbia [5] and *Dragmacidon* sp. (Axinellida) from the Bahamas [6], as well as from the ascidian *Didemnum cadidum* from shallow waters in southern California [7]; recently they were also obtained by total synthesis [8]. By bearing a pyrazinone central nucleus and a 2-aminoimidazole unit at an ethyl chain of one of the indole moieties [9], dragmacidin d, isolated from *Spongosorites* sp. from deep-sea of the Grenadines, represents a peculiar variant to the dragmacidins.

We report here on nortopsentin D[1]) (**5**), a novel structural variant to alkaloids of the above families, and on a putative biogenetic precursor, *i.e.*, 4-methyl-1*H*-imidazol-2-amine (**6**). They were isolated, together with **7**, from the axinellid sponge *Dragmacidon* sp. from deep waters south of New Caledonia. Although **5** surprisingly proved inactive on tumoural cells, a simple chemical transformation afforded highly cytotoxic **11**.



5[1])



6 R=Me
7 R=H

2. Results and Discussion. – The composition $C_{23}H_{17}Br_2N_7O$ for nortopsentin D (**5**), implying 18 double-bond equivalents, was secured from the FAB-MS cluster *m/z* 570/568/566 and NMR data. Thus, in the ^{13}C-NMR spectra in $(CD_3)_2SO$ (*Table 1*), signals emerged as separate, though often broad resonances while in CD_3OD, part of the signals proved too broad to be detectable. Imidazole tautomerism was reflected in the doubling of many ^{1}H-NMR signals in a 85:15 integration ratio in either CD_3OD or $(CD_3)_2SO$ [3] (*Table 2*); collapse to a single series of signals was observed on addition of a stoichiometric amount of CF_3COOH (*Table 2*). That there are six exchangeable protons was revealed by exchange with D_2O. The two 6-bromo-1*H*-indole moieties, C(3)-bound to either a sp^2 or sp^3 C-atom, were inferred from typical δ and *J* data in the 1D- and 2D-NMR spectra (*Tables 1* and *2*), from hetero correlation (^3J in HMBC) of H–C(2‴) with C(5), and from HR-EI-MS on fragment ions *m/z* 195 and 220 (*Exper. Part*).

The presence of the 2-amino-4-methyl-1*H*-imidazole appendage in **5** finds support in both ^{1}H,^{13}C HMBC correlations, in particular ^3J(C,H) of H–N(1″) with C(3), and HR-EI-MS on fragment ion *m/z* 139 of methyl derivative **11**. The lactam nature of the central unit of **5** rests on NOE enhancement between *Me*–N(1)/*H*–C(2‴) and on the hetero correlations *Me*–N(1)/C(5)/C(2) for the *N*-methyl derivatives which were formed

[1]) Arbitrary numbering is for convenience; for systematic names, see *Exper. Part.*

Table 1. *^{13}C-NMR Data for Nortopsentin D* (**5**) *and Its Methyl Derivatives* **8**–**11**[1]). In CD_3OD, unless otherwise stated. δ in ppm and J in Hz.

	5[a][b])	**8**	**9**	**10**	**11**[c])
C(2)	181.62 (*s*)	180.36 (*s*)	181.91 (*s*)[d])	180.75 (*s*)	179.593 (*s*)
C(3)	69.36 (*s*)	[e])	71.25 (*s*)	[e])	69.55 (*s*)
C(5)	156.75 (*s*)	[e])	160.76 (*s*)	[e])	158.87 (*s*)
C(2′)	124.49 (*d*, *J* = 185)	125.43 (*d*)[f])	125.91 (*d*)[f])	125.61 (*d*)[f])	128.60 (*d*)
C(3′)	112.06 (*s*)	110.36 (*s*)	113.56 (*s*)	113.59 (*s*)	110.54 (*s*)
C(3′a)	124.39 (*s*)	126.49 (*s*)[g])	126.81 (*s*)[g])	127.46 (*s*)[g])	124.31 (*s*)
C(4′)	121.43 (*d*, *J* = 163)	123.41 (*d*)[f])	122.81 (*d*)	122.78 (*d*)[f])	121.13 (*d*)
C(5′)	121.95 (*d*, *J* = 165)	123.76 (*d*)[f])	124.20 (*d*)	124.33 (*d*)[f])	123.48 (*d*)[f])
C(6′)	114.24 (*s*)	115.60 (*s*)[h])	116.99 (*s*)	[e])	116.19 (*s*)
C(7′)	114.36 (*d*, *J* = 167)	115.74 (*d*)[i])	116.06 (*d*)	116.10 (*d*)	112.99 (*d*)
C(7′a)	137.58 (*s*)	139.79 (*s*)[j])	139.75 (*s*)[h])	139.85 (*s*)	138.35 (*s*)
C(2″)	145.98 (*s*)	148.12 (*s*)	148.66 (*s*)	148.62 (*s*)	147.47 (*s*)
C(4″)	118.74 (*s*)	117.21 (*s*)	117.96 (*s*)	117.01 (*s*)	126.19 (*s*)
C(5″)	119.23 (*s*)	[e])	121.16 (*s*)	[e])	125.67 (*s*)
CH_3–C(4″)	9.53 (*q*)	9.78 (*q*)	9.89 (*q*)	9.88 (*q*)	10.39 (*q*)
C(2‴)	130.92 (*d*, *J* = 187)	131.95 (*d*)	131.07 (*d*)	130.60 (*d*)	133.22 (*d*)
C(3‴)	104.78 (*s*)	[e])	105.94 (*s*)	105.41 (*s*)	103.40 (*s*)
C(3‴a)	124.04 (*s*)	126.37 (*s*)[g])	125.84 (*s*)[g])	125.89 (*s*)[g])	123.55 (*s*)
C(4‴)	123.15 (*d*, *J* = 167)	124.62 (*d*)[f])	124.68 (*d*)	124.78 (*d*)[f])	123.51 (*d*)[f])
C(5‴)	123.96 (*d*, *J* = 167)	125.27 (*d*)[f])	125.59 (*d*)[f])	126.22 (*d*)[f])	125.25 (*d*)
C(6‴)	115.35 (*s*)	116.59 (*s*)[h])	116.99 (*s*)	117.01 (*s*)	117.07 (*s*)
C(7‴)	114.82 (*d*, *J* = 167)	116.00 (*d*)[i])	116.20 (*d*)	114.64 (*d*)	112.99 (*d*)
C(7‴a)	137.62 (*s*)	139.62 (*s*)[j])	139.18 (*s*)[h])	139.85 (*s*)	137.90 (*s*)
X	–	–	–	–	41.26 (*q*)
R^1	–	–	30.01 (*q*)	29.99 (*q*)	29.70 (*q*)
R^2	–	–	–	–	33.21 (*q*)
R^3	–	32.33 (*q*)	32.57 (*q*)	32.59 (*q*)	34.08 (*q*)
R^4	–	30.08 (*q*)	30.27 (*q*)	31.19 (*q*)	32.48 (*q*)
R^5	–	–	–	34.07 (*q*)	33.72 (*q*)

[a]) Data of the major tautomer of **5**; assignments based also on selective heteronuclear decoupling; ^1J(C,H) from the fully coupled spectrum. [b]) In $(CD_3)_2SO$. [c]) In $CDCl_3$. [d]) 179.68 ppm in $(CD_3)_2SO$. [e]) These resonance could not be detected. [f])[g])[h])[i])[j]) Data interchangeable within the same column.

Table 2. *^{1}H-NMR Data for Nortopsentin D* (**5**) *and Its Methyl Derivatives* **8**–**11**[1]). In CD_3OD, unless otherwise stated. δ in ppm, J in Hz.

	5[a][b][c])	**5**[a][d][e])	**8**	**9**	**10**	**11**[f])
H–C(2′)	7.35 (*d*, *J* = 2.4)	7.41 (*d*, *J* = 2.7)	7.29 (br. *s*)	7.28 (*s*)	7.28 (*s*)	7.27 (*s*)
H–C(4′)	7.56 (*d*, *J* = 8.4) [7.58]	7.50 (*d*, *J* = 8.7)	7.57 (*d*, *J* = 8.7)	7.46 (*d*, *J* = 8.6)	7.47 (*d*, *J* = 8.7)	7.34 (*d*, *J* = 8.6)
H–C(5′)	7.15 (*dd*, *J* = 8.4, 1.5) [7.14]	7.16 (*dd*, *J* = 8.7, 1.8)	7.09 (*dd*, *J* = 8.7, 1.8)	7.12 (*dd*, *J* = 8.6, 1.8)	7.12 (*dd*, *J* = 8.6, 1.8)	7.15 (*dd*, *J* = 8.6, 1.5)
H–C(7′)	7.61 (*d*, *J* = 1.5) [7.62]	7.62 (*d*, *J* = 1.8)	7.54 (*d*, *J* = 1.8)	7.58 (*d*, *J* = 1.8)	7.59 (*d*, *J* = 1.8)	7.45 (*d*, *J* = 1.5)
CH_3–C(4″)	2.02 (*s*) [1.96]	1.95 (*s*)	1.99 (*s*)	2.10 (*s*)	2.10 (*s*)	2.18 (*s*)

Table 2 (cont.)

	5[a][b][c]	5[a][d][e]	8	9	10	11[f]
H–C(2‴)	8.18 (*d*, *J* = 2.7)	8.35 (br. *s*)	8.00 (br. *s*)	8.14 (*s*)	8.15 (*s*)	8.17 (*s*)
H–C(4‴)	8.25 (*d*, *J* = 8.4) [8.30]	8.26 (*d*, *J* = 8.7)	8.26 (*d*, *J* = 8.7)	8.23 (*d*, *J* = 8.7)	8.26 (*d*, *J* = 8.7)	8.18 (*d*, *J* = 8.7)
H–C(5‴)	7.34 (*dd*, *J* = 8.4, 1.5) [7.41]	7.39 (*dd*, *J* = 8.7, 1.5)	7.26 (*dd*, *J* = 8.7, 1.8)	7.28 (*dd*, *J* = 8.7, 1.8)	7.33 (*dd*, *J* = 8.7, 1.8)	7.32 (*dd*, *J* = 8.7, 1.7)
H–C(7‴)	7.73 (*d*, *J* = 1.5) [7.78]	7.75 (*d*, *J* = 1.5)	7.62 (*d*, *J* = 1.8)	7.68 (*d*, *J* = 1.8)	7.74 (*d*, *J* = 1.8)	7.54 (*d*, *J* = 1.7)
X	–	–	–	–	–	2.99 (*s*)
R^1	–	–	–	3.48 (*s*)	3.50 (*s*)	3.53 (*s*)
R^2	–	–	–	–	–	3.73 (*s*)
R^3	–	–	3.27 (*s*)	3.26 (*s*)	3.26 (*s*)	3.44 (*s*)
R^4	–	–	3.40 (*s*)	3.40 (*s*)	3.42 (*s*)	3.67 (*s*)
R^5	–	–	–	–	3.92 (*s*)	3.91 (*s*)

[a]) In $(CD_3)_2SO$. [b]) Data of major tautomer of 85:15 mixture; in brackets, not overlapped signals for the minor tautomer. [c]) H–N(1′): 11.45 (br. *d*, *J* = 2.4) [11.40]; H–N(1″): 11.82 (br. *s*); H_2N–C(2″): 6.93 (br. *s*) [6.98]; H–N(1‴): 12.00 (br. *s*). [d]) With added CF_3COOH in stoichiometric amount. [e]) H–N(1′): 11.43 (br. *s*); H–N(1″): 12.09 (br. *s*); H_2N–C(2″): 7.05 (br. *s*); H–N(1‴): 12.93 (br. *s*). [f]) In $CDCl_3$.

from **5** on treatment with MeI/K_2CO_3 in the kinetic sequence **8**, **9**, **10**, and **11**[2]) (*Scheme*). That the lactam moiety is actually a 2,4,4-trisubstituted 1*H*-imidazol-5(4*H*)-one is supported by *a*) δ(C) 181.62 ppm for C(2)=O of **5**, slightly upfield shifted on methylation at N(1) (*Table 1*) [11] and *b*) IR stretching at 1730 and 1610 cm^{-1} in both **5** and **11**, which are typical of nonconjugated C=O and C=N groups, respectively [12][3]). The attribution of the *m*/*z* 568 FAB-MS signal to $[M + H]^+$ of **5** is in accordance with the observation of the highest-mass FAB-MS clusters for **8**–**11** (*Exper. Part*) which require the presence of two, three, four, and seven Me groups, respectively. Chirality at the C(3) centre of **5** was difficult to establish[4]), but was supported by a *Cotton* effect observed for derivative **11** (*Exper. Part*).

The biogenesis of **5** can be conceived *via* nucleophilic addition of 4-methyl-1*H*-imidazol-2-amine (**6**) to an elusive intermediate epoxy derivative of nortopsentin A (**1**) [1]. This proposal finds support in the co-occurrence of **6** with **1** in *Dragmacidon* sp. Although 1*H*-imidazol-2-amine (**7**) was also isolated from *Dragmacidon* sp. in abundance[5]), no

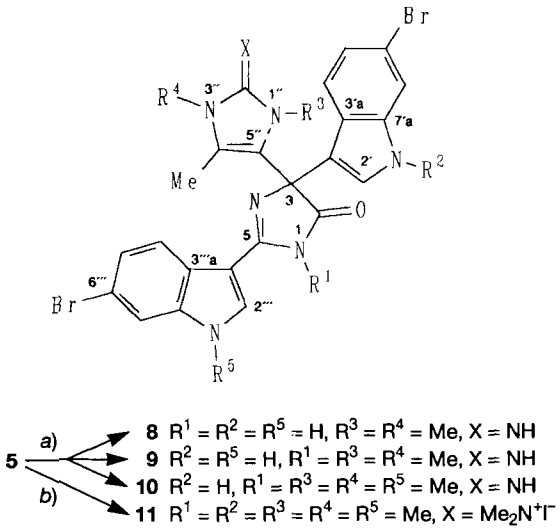
[2]) The C(2″)=NH form for derivatives **8**–**11** is supported by NOE enhancements in the couples Me–N(3″)/Me–C(4″) of **9** and **10** and *Me*–C(1″)/*H*–C(2′) of **10**. This is consistent with the presence in **5** of a 2-amino group at the imidazole ring which rationalizes both the increased reactivity of alkylating agents at the pyridine-like N-atom and the decreased reactivity at the amino group [10].

[3]) According to literature analogies, a nonconjugated C=O form is preferred to a conjugated form; the position of the tautomeric equilibrium is independent of the solvent nature though it depends on the nature of the substituents [13].

[4]) Because of their light-absorbing reddish solutions, difficulties were experienced in obtaining chiroptical data for either compound **5** or its methyl derivatives **8**–**11**. Only with very dilute solutions, a *Cotton* effect could be observed for **11** (*Exper. Part*).

[5]) Probably deriving from arginine metabolism, **7** was previously isolated from both the terrestrial actinomycete *Streptomyces eurocidicus* [14] and the marine sponge *Reniera cratera* [15].

Scheme



a) MeI, K_2CO_3, acetone, r.t., 4 h. *b*) MeI, K_2CO_3, acetone, r.t., 3 days.

trace was found of the corresponding analogue of **5**; this suggests a nucleophilicity-enhancing role of the Me substituent of **6** [16].

The 2-amino-imidazole moiety occurs frequently in marine natural products [17] and is of special interest in medicinal chemistry [18]. Nortopsentin D (**5**) and its derivatives **8** and **10**, in spite of possessing a biologically active appendage like the 2-aminoimidazole group [18] on a skeleton equivalent to potently antitumoural molecules like the topsentins and nortopsentins [1] [2] [19], proved inactive on KB tumoural cells *in vitro*. They also showed little antibacterial activity on *Staphylococcus aureus*. The introduction of the last three methyl groups, like in **11**, though not improving on either antibacterial (*S. aureus*) or antifungal (*Candida albicans*) activity, led to high cytotoxicity on KB cell lines, (EC_{50} = 0.014 μg/ml). What the role may be of permethylation, as in **11**, in affording high cytotoxicity is difficult to guess on present basis. Perhaps the amphiphylic nature of **11** is a determinant factor.

We thank Prof. *C. Lévi*, Musée National d'Histoire Naturelle, Paris, for the taxonomic identification and Mr. *M. Rossi* and *A. Sterni* for skilled technical contributions in product isolation and mass spectra, respectively. This work was carried out within the collaborative *ORSTOM-CNRS Program on Marine Substances of Biological Interest*. The work in Trento was supported by *MURST* (Progetti 40%) and *CNR*, Roma.

Experimental Part

1. *General.* All evaporations were carried out under reduced pressure. Yields are given on reacted reagents. M.p.: *Kofler* hot-stage microscope (uncorrected). Flash chromatography (FC): *Merck Si-60*, 15–25 μm. TLC: *Merck* silica gel *60* PF_{254} plates. Reversed-phase HPLC: 25 × 1 cm columns with *Merck LiChrospher RP18* (7 μm); UV monitoring at 254 nm; solvent flux 5 ml min^{-1}. Polarimetric data: *Jasco-DIP-181* polarimeter; $[\alpha]_D$ values in 10^{-1} deg cm^3 g^{-1}. UV: *Perkin-Elmer-Lambda-3* spectrophotometer. CD: *Jasco-J710* spectropolarimeter (λ_{max} in

nm, ε in $mol^{-1} \cdot l \cdot cm^{-1}$. IR: *Philips-Pye-Unicam-SP3-200S* spectrometer. NMR: *Varian-XL-300* spectrometer, ^1H at 300 MHz, ^{13}C at 75.4 MHz; δ values in ppm, in $CDCl_3$ rel. to internal $SiMe_4$ (= 0 ppm), in $(CD_3)_2SO$ at 30° rel. to the solvent (δ(H) 2.49, δ(C) 39.50), and in CD_3OD rel. to the solvent (δ(H) 3.30, δ(C) 49.30); J values in Hz; multiplicities from DEPT [20]; $^1H,^1H$ from COSY [21]; $^1H,^{13}C$ assignments from one-bond [22a] and long-range COSY [22b] or $^{13}C,^1H$-NMR by inverse detection shift correlation experiments [23a], carried out with $^nJ(C,H) = 4$ or 7 Hz, using a dedicated probe [23b] (HMBC = heteronuclear multiple bond coherence spectroscopy); differential NOE data (obtained with 5 s pre-irradiation) are reported as 'irradiated proton → NOE on the observed proton(s)'. Mass spectra (*m/z*; rel. %): *Kratos-MS80* mass spectrometer with home-built computerized acquisition data; *Vacumetrics DIP* gun for FAB.

2. *Collection and Isolation.* The sponge (collections R1446-623M and R1446-668M) was taken by dredging at 300 m depth on the *Monts Sous Marin* south of New Caledonia. It was determined taxonomically by Prof. *C. Lévi* who retains a voucher specimen (Musée National d'Histoire Naturelle, Paris). The sponge was immediately frozen after collection and then freeze-dried (33 g dry weight). Extraction with MeOH and evaporation of the org. extract gave 3.48 g of residue which was subjected to FC (hexane/AcOEt, then AcOEt/MeOH gradient elution), collecting 17 fractions of 100 ml each. Pure **5** (0.23 g) was obtained from evaporation of *Fr. 7*, eluted with AcOEt/MeOH 3:2. The residue from evaporation of *Fr. 8* (0.53 g) was a mixture **5**/**6**/**7** 2:1:1. A small amount of *Fr. 8* was separated on a column of *Dowex-X8* (200–400 mesh, chloride form, *Bio-Rad*) eluting first with H_2O (→ **6**/**7**) and then with MeOH (→ pure **5**). A 2:5 mixture **6**/**7** was also obtained by HPLC (MeCN/H_2O 2:3, t_R 2.0 min) of a small amount of the crude residue.

3. *Nortopsentin D* (= *4-(2-Amino-4-methyl-1*H*-imidazol-5-yl)-2,4-bis(6-bromo-1*H*-indol-3-yl)-1*H*-imidazol-5(4*H*)-one*; **5**). Amorphous solid. UV (MeOH): 360 (15400), 294 (22000), 276 (28000). UV (MeOH, $5 \cdot 10^{-3}$ M HCl): 334 (21000), 276 (28000). UV (MeOH, $4 \cdot 10^{-3}$ M NaOH): 352 (6000), 283 (38000). IR (KBr): 3250, 1730, 1680, 1610, 1550, 1450, 1400, 1220. HMBC ($(CD_3)_2SO$): H–N(1′) → C(2′); H–C(2′) → C(3′); H–C(4′) → C(6′), C(7′a); H–C(5′) → C(6′), C(7′); H–C(7′) → C(3′a), C(6′); Me–C(4″) → C(4″). EI-MS: 330, 332 (4, 4); 277, 279 (2, 2); 220, 222 (72, 72); 195, 197 (32, 32); 141 (46); 116 (29); 96 (30); 79, 81 (8, 8); 42 (28). HR-EI-MS: 330.0079 ± 0.0040 ($C_{14}H_{11}{}^{79}BrN_4O^+$, calc. 330.0116); 219.9650 ± 0.0030 ($C_9H_5{}^{79}BrN_2^+$, calc. 219.9636); 194.9672 ± 0.0030 ($C_8H_6{}^{79}BrN^+$, calc. 194.9683). FAB-MS (glycerol, H^+, matrix): 566, 568, 570 (4, 8, 4, $[M + H]^+$); 371, 373 (12, 12).

4. *4-Methyl-1*H*-imidazol-2-amine* (**6**). ^1H-NMR ($(CD_3)_2SO$): 6.53 (*q*, $J = 0.9$, H–C(5)); 2.05 (*d*, $J = 0.9$, Me–C(4)); 7.26 (br. *s*, NH_2); 11.58 (br. *s*, NH). ^{13}C-NMR (CD_3OD): 148.72 (*s*, C(2)); 124.74 (*s*, C(4)); 110.24 (*d*, C(5)); 10.08 (*q*, Me). EI-MS: 97 (26, $M^{+\cdot}$), 96 (17). HR-EI-MS: 97.0608 ± 0.0040 ($C_4H_7N_3^+$, calc. 97.0639). FAB-MS (glycerol, H^+, matrix): 98 ($[M + H]^+$).

5. *1*H*-Imidazol-2-amine* (**7**). ^1H-NMR ($(CD_3)_2SO$): 6.83 (*s*, H–C(4), H–C(5)); 7.26 (br. *s*, NH_2); 11.58 (br. *s*, NH). ^{13}C-NMR (CD_3OD): 149.05 (*s*, C(2)); 114.48 (*s*, C(4), C(5)). EI-MS: 83 (100, $M^{+\cdot}$). HR-EI-MS: 83.0483 ± 0.0030 ($C_3H_5N_3^+$, calc. 83.0483). FAB-MS (glycerol, H^+, matrix): 84 ($[M + H]^+$).

6. *Methylation of* **5**. To a soln. of **5** (32.4 mg, 0.057 mmol) in Me_2CO (2.5 ml) were added K_2CO_3 and an excess of MeI (0.5 ml). The mixture was stirred at r.t. until all **5** had disappeared (4 h); then it was filtered and evaporated. The residue was subjected to reversed-phase HPLC (MeCN/aq. buffer 55:45, pH 2.0): three fractions. Each fraction was neutralized with $NaHCO_3$ and cooled at −20°, upon which the org. phase was separated. The latter was evaporated: **8** (t_R 4.3 min; 9.0 mg, 26%), **9** (t_R 5.9 min; 15.9 mg, 46%), and **10** (t_R 11.0 min; 9.5 mg, 27%), resp.

Compound **5** (27.2 mg, 0.048 mmol) was treated as above for 3 days instead of 4 h. Similar workup and HPLC purification (MeCN/aq. buffer 3:1, pH 2.0) led to a single compound **11** (t_R 6.2 min; 31.6 mg, 83%).

*2,4-Bis(6-bromo-1*H*-indol-3-yl)-4-(2,3-dihydro-2-imino-1,3,5-trimethyl-1*H*-imidazol-4-yl)-1*H*-imidazol-5(4*H*)-one* (**8**): IR (KBr): 3410, 1715, 1650, 1620, 1550, 1450. FAB-MS (glycerol, H^+, matrix): 594, 596, 598 (9, 18, 9, $[M + H]^+$); 516, 518 (3, 3).

*2,4-Bis(6-bromo-1*H*-indol-3-yl)-4-(2,3-dihydro-2-imino-1,3,5-trimethyl-1*H*-imidazol-4-yl)-1-methyl-1*H*-imidazol-5(4*H*)-one* (**9**): IR (KBr): 3410, 1715, 1650, 1620, 1550, 1450. NOE (CD_3OD): 3.48 (Me–N(1)) → 8.14 (H–C(2″)); 2.10 (Me–C(4″)) → 3.40 (Me–N(3″)). EI-MS: 234, 236 (0.5, 0.5); 220, 222 (2, 2); 195, 197 (4, 4). FAB-MS (glycerol, H^+, matrix): 608, 610, 612 (24, 48, 24, $[M + H]^+$); 530, 532 (9, 9).

*4-(6-Bromo-1*H*-indol-3-yl)-2-(6-bromo-1-methyl-1*H*-indol-3-yl)-4-(2,3-dihydro-2-imino-1,3,5-trimethyl-1*H*-imidazol-4-yl)-1-methyl-1*H*-imidazol-5(4*H*)-one* (**10**): NOE (CD_3OD): 3.92 (Me–N(1‴)) → 8.15 (H–C(2‴)), 7.74 (H–C(7‴)); 3.50 (Me–N(1)) → 8.15 (H–C(2‴)); 3.26 (Me–N(1″)) → 7.28 (H–C(2′)); 2.10 (Me–C(4″)) → 3.42 (Me–N(3‴)). EI-MS: 234, 236 (0.8, 0.8); 209, 211 (0.3, 0.3). FAB-MS (glycerol, H^+, matrix): 622, 624, 626 (3, 6, 3, $[M + H]^+$); 544, 546 (2, 2).

*4-[2,4-Bis(6-bromo-1-methyl-1*H*-indol-3-yl)-4,5-dihydro-1-methyl-5-oxo-1*H*-imidazol-4-yl]-*N,N*,1,3,5-pentamethyl-1*H*-imidazol-2(3*H*)-iminium Iodide* (**11**): M.p. 172° (dec.). $[\alpha]_D^{25} = +0.0$; $[\alpha]_{365}^{25} = -17$; ($c = 0.05$, MeOH). UV (MeOH): 288 (24000). CD (MeOH; $\Delta\varepsilon(\lambda)$): −0.73 (298), +0.64 (264). IR (KBr): 3430, 2940, 1735, 1610, 1470, 1370. NOE ($CDCl_3$): 3.91 (Me−N(1‴)) → 8.17 (H−C(2‴)), 7.54 (H−C(7‴)); 3.73 (Me−N(1′)) → 7.27 (H−C(2′)), 7.45 (H−C(7′)); 3.67 (Me−N(3″)) → 2.99 (Me_2N=C(2″)), 3.53 (Me−N(1)) → 8.17 (H−C(2‴)); 3.44 (Me−N(1″)) → 2.99 (Me_2N=C(2″)). ^{1}H,^{13}C-COSY (only the most significant hetero correlations are reported): C(2) → *Me*−N(1); C(5) → *Me*−N(1); C(2′) → *Me*−N(1′); C(5″) → *Me*−C(4″); C(4″) → Me−N(3″). EI-MS: 413, 415 (7, 7); 234, 236 (90, 90); 219, 221 (6, 6); 209, 211 (52, 52); 139 (58). HR-EI-MS: 139.1105 ± 0.0030 ($C_7H_{13}N_3^+$, calc. 139.1109). FAB-MS of **11**·(glycerol matrix, CF_3COOH): 664, 666, 668 (11, 22, 11, M^+ of org. cation); 585, 587 (3, 3).

7. *Biological Assays.* By known methodologies [24], the inhibition halo around the disk on cultures of *Staphylococcus aureus* for compounds **5**, **8**, and **10**, at 100 μg/disk, was 10, 12, and 9 mm, resp. Cytotoxicity towards KB cell lines for these compounds, at 10 μg/ml, was also negligible, 20, < 15, and < 15%, resp. Introduction of the last three methyl groups, as in **11**, while not improving on antibacterial activity, led to high cytotoxicity ($EC_{50} = 0.014$ μg/ml from dilution experiments) on KB cell lines.

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