(+)-Agelasine D: Improved Synthesis and Evaluation of Antibacterial and Cytotoxic Activities[#]

Anders Vik,[†] Erik Hedner,[‡] Colin Charnock,[§] Ørjan Samuelsen,[⊥] Rolf Larsson,[∥] Lise-Lotte Gundersen,*[,†] and Lars Bohlin[‡]

Department of Chemistry, University of Oslo, PO Box 1033 Blindern, N-0315 Oslo, Norway, Division of Pharmacognosy, Department of Medicinal Chemistry, Biomedical Centre, Uppsala University, PO Box 574, S-751 23, Sweden, Faculty of Health Sciences, Oslo University College, PO Box 4 St. Olavs plass, N-0130 Oslo, Norway, Department of Medical Microbiology, University Hospital of North Norway, Tromsø, Norway, and Division of Clinical Pharmacology, Department of Medicinal Sciences, Uppsala Academic Hospital, S-751 85 Uppsala, Sweden

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An improved synthesis of (+)-agelasine D (**10**) from (+)-manool is reported together with cytotoxic and antibacterial data for agelasine D and structurally close synthetic analogues. These compounds display a broad spectrum of antibacterial activities including effects on *M. tuberculosis* and Gram-positive and Gram-negative bacteria (both aerobes and anaerobes). They exhibit profound cytotoxic activity against several cancer cells, including a multidrug-resistant cell line. (+)-Agelasine D (**10**) has been isolated earlier from a marine sponge (*Agelas* sp.).

Marine sponges are rich sources of natural products with a range of biological activities.[1] Agelasines are isolated from marine sponges (*Agelas* spp.), and they are associated with bioactivities such as antimicrobial and cytotoxic effects, as well as contractive responses of smooth muscles and inhibition of Na- and K-ATPase.[2] More than 10 agelasines are currently known, but only agelasines A,[3] B,[4] D,[5] E,[6] and (±)-agelasine F[7] (Figure 1) have been synthesized. We recently published the first synthesis of (+)-agelasine D (**10**).[5] Even though the reaction sequence constitutes only a few steps from commercially available (+)-manool, the total yield of the target compound is less than desired due to moderate regio- or stereoselectivity in some steps. We herein report an improved conversion of (+)-manool to (+)-agelasine D (**10**) and also the first antibacterial and cytotoxic data for this natural product and its synthetic intermediates.

Results and Discussion

Synthesis. In our previously reported synthesis of (+)-agelasine D,[5] (+)-manool was converted directly into the allylic bromide **4** (Scheme 1). However, an inseparable mixture of the *E*- and *Z*-isomers of the bromide had to be used in the alkylation step (conversion of **6a** to **9a**, Scheme 3). Pure (*E*)-**9a** was isolated in modest yield after crystallization. Therefore, we sought a method for converting (+)-manool to pure (*E*)-**4**. Even though iodination of tertiary allylic acetates with Ph_3PI_2 occurs with complete *E*-selectivity, this reaction could not be used for our purpose since isomerization of exocyclic double bonds readily takes place under these conditions.[8] Geometrically pure primary allylic bromides can be formed by Pd-catalyzed rearrangement of tertiary allylic esters, such as the acetates of linalool and geranyllinalool, followed by ester hydrolysis and bromination.[6] When manool acetate (**1**) was subjected to rearrangement catalyzed by $PdCl_2(MeCN)_2$ at ambient temperature, full conversion of the starting material took place in less than an hour (Scheme 1), whereas the same rearrangement of geranyllinalyl acetate required more than 1 day.[6] At ambient temperature the *E*:*Z* ratio of **2** was 87:13. The ratio was somewhat improved by lowering the temperature, and a 92:8 mixture of (*E*)- and (*Z*)-**2** was obtained at 0 °C. Decreasing the reaction temperature below 0 °C did not improve selectivity further. The acetate was hydrolyzed to give, after chromatography, pure (*E*)-**3**, which was converted to the alkylating agent **4** by treatment of PBr_3. Compound **3** is found in nature and referred to as (+)-copalol or *anti*-copalol. To the best of our knowledge, the transformation of (+)-manool into (+)-copalol (**3**), described herein, is the most convenient synthesis of this natural product published to date.[9]

A second drawback with our first (+)-agelasine D synthesis is the low regioselectivity in the N-7 alkylation step (conversion of **6a** to **9a**, Scheme 3).[5] Introduction of an alkyl group in the 7-position of an N-9-alkylated adenine derivative requires a directing group at N^6. A methoxy group is commonly used, but substantial amounts of N^6-alkylated products are generally formed together with the desired 7,9-dialkylpurinium salt.[4−7,10] Also acyl groups have been suggested as directing groups.[11] We introduced the acetyl, benzoyl, and *tert*-butanoyl functionality at N^6 of 9-methyladenine. All compounds reacted with excess benzyl bromide in a highly regioselective manner to give 7,9-dialkylpurinium salts, but the isolated yields were often quite modest due to decomposition during workup and purification. Instead we chose to use alkoxy directing groups, and we assumed that increased sterical hindrance at N^6 would favor N-7 alkylation. The 6-chloropurine **5** was reacted with different N-alkoxyamines to give compounds **6** (Scheme 2). In DMSO-d_6 solution compounds **6** exist mainly as the tautomer shown in Scheme 2. Compounds **6a**−**c** were isolated in acceptable yields. Syntheses of compounds **6** with R = −Ph, $-CPh_3$, or −TBDMS were not successful. In these latter cases, the $RONH_2$ reagents used were of limited stability at the temperatures generally required for nucleophilic substitution on compound **5**. When compounds **6a**−**c** reacted with benzyl bromide, it was confirmed that the regioselectivity in the alkylation was highly dependent on the sterical shielding of N^6 (Table 1). The *tert*-butoxy group was found to be an excellent directing group and used in the agelasine D synthesis below. Compound **6b** was reacted with copalyl bromide **4** to give the desired product **9b** in high yield (Scheme 3). The alkylation occurred with complete regioselectivity, as judged by ^{1}H NMR of the crude product. Agelasine D (**10**) was isolated after reductive cleavage of the directing group.

Antibacterial Activity. Many natural products from marine sponges are associated with potent antibacterial activities.[1c] Agelasines B and F (Figure 1) exhibit activity against several types of bacteria.[2c,g] Agelasine F is even reported to inhibit *Mycobacterium tuberculosis*.[12] On the other hand, *epi*-agelasine C is found to be inactive against several microorganisms including *Staphylococcus aureus* and *Escherichia coli*.[2e] The same is true for the agelasine

[#] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

* Corresponding author. Tel: +47 22 857019. Fax: +47 22 855507. E-mail: l.l.gundersen@kjemi.uio.no.

[†] University of Oslo.

[‡] Uppsala University.

[§] Oslo University College.

[⊥] University Hospital of North Norway.

[∥] Uppsala Academic Hospital.

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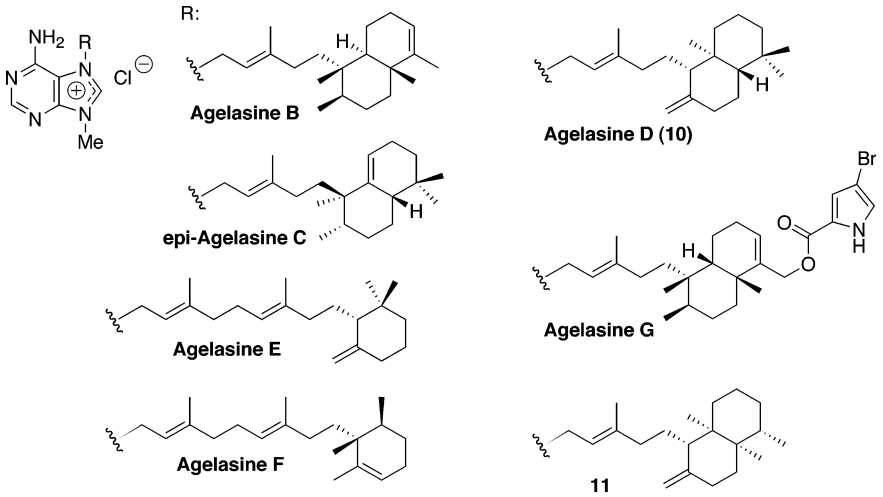
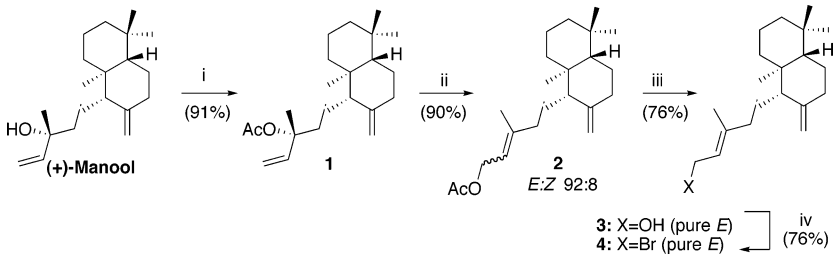


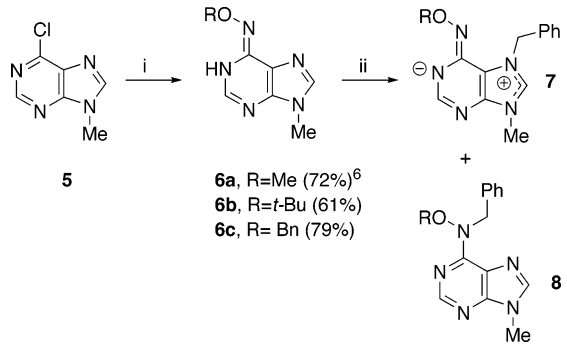
Figure 1. Structures of agelasines discussed in the text.

Scheme 1[a]



[a] (i) Ac_2O, DMAP, Et_3N, THF, Δ; (ii) $PdCl_2(MeCN)_2$, THF, 0 °C; (iii) K_2CO_3, MeOH; (iv) PBr_3, pyridine, Et_2O, 0 °C.

Scheme 2[a]



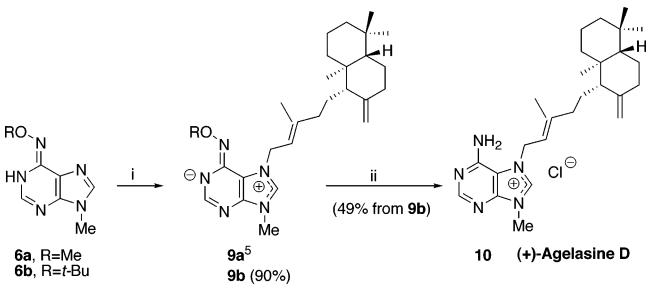
[a] (i) $RONH_3Cl$ or $RONH_2$ Et_3N, *n*-BuOH, Δ; (ii) BnBr, DMA, 50 °C.

Table 1. Regioselectivity in Alkylation of Compounds **6**

R	ratio **7**:**8**[a]	yield (%) **7**[b]	yield (%) **8**[b]
Me	3:2	55, **7a**	30, **8a**
t-Bu	9:1	86, **7b**	9, **8b**
Bn	4:1	72, **7c**	13, **8c**

[a] From ^1H NMR of the crude product. [b] Isolated yields.

Scheme 3[a]



[a] (i) Comp. **4**, DMA, 50 °C; (ii) Zn, AcOH, MeOH, H_2O, 75 °C.

11.[2g] We have demonstrated profound antimycobacterial activity for several synthetic analogues of agelasines, but only moderate activity for agelasine E.[6] It is claimed that other agelasines, including agelasine D, are antimicrobial, but no details regarding microorganisms nor quantitative activity were reported.[2a] Synthetically prepared (+)-agelasine D (**10**) and the N^6-alkoxy derivatives **9a**,**b** were screened against Gram-positive and Gram-negative bacteria, as well as *M. tuberculosis*, and the results are summarized in Table 2. The compounds examined were found to exhibit relatively broad antibacterial spectra. Highest activities were found against the Gram-positive bacteria *S. aureus* and *S. pyogenes*. The *Bacteroides fragilis* group of organisms are the most commonly isolated anaerobes in clinical settings. Species of this group have shown an increase in resistance to most of the antimicrobial agents traditionally used for treating anaerobic infections.[13] The MIC values for compounds **9** and **10** were comparable with those obtained using metronidazole, which has been the drug of choice for preventing and treating infections by the *B. fragilis* group species for 40 years.[14] Agelasine analogues of low cytotoxicity might thus have potential as antibiotics for treatment of infections by these organisms. All compounds were profound inhibitors of *M. tuberculosis* at 6.25 μg/mL. Only minor differences in activity between agelasine D and the alkoxyoxy derivatives **9** were found, even though the alkoxy group appears to be very important for antimycobacterial activity in many agelasine analogues.[6] The *tert*-butoxy derivative of agelasine D, **9b**, was somewhat less active against *S. aureus* and *E. coli*, compared to compounds **9a** and **10**, but activities against *M. tuberculosis*, *B. fragilis*, and *B. thetaiotaomicron* were comparable to those found for compound **9a**.

Cytotoxicity. Agelasine G is cytotoxic against murine lymphoma cells,[15] but cytotoxicity data for other agelasines are, to the best of our knowledge, not available in the literature. We chose to screen agelasine D (**10**) and the analogues **9a**,**b** against four human tumor cell lines: lymphoma (U-937 GTB), myeloma (RPMI 8226/s), leukemia (CEM/s), and renal cells (ACHN). The latter is a multidrug-resistant cell line, whereas the others are regarded as

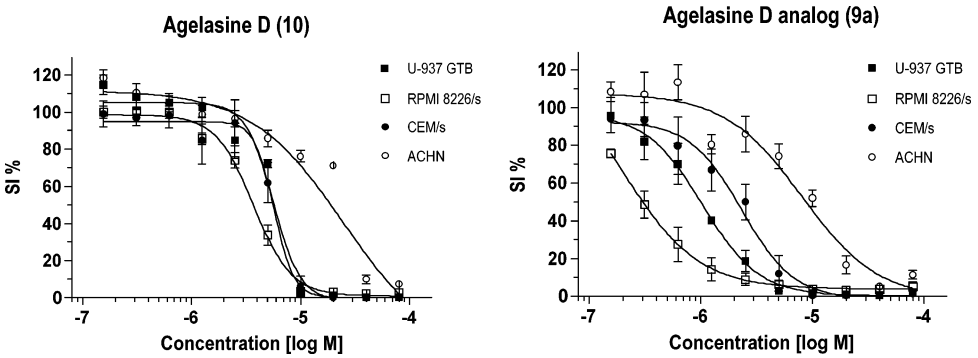


Figure 2. Survival index (SI) curves of agelasine **10** (left) and analogue **9a** (right) at 10 concentrations on the human cancer cell lines U-937 GTB, RPMI 8226/s, CEM/s, and ACHN. Data points are mean ± SEM of triplicates values, calculated using nonlinear regression in GraphPad Prism 4 (GraphPad Software, San Diego, CA).

Table 2. Antibacterial Activity of Compounds **9a**, **9b**, and Agelasine D (**10**) and Clinically Used Drugs against *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacteroides fragilis*, and *Bacteroides thetaiotaomicron*

	% inhibition *M. tuberculosis* $H_{37}Rv$	MIC (μg mL^{-1})							
compound	at 6.25 mg/mL	*M. tuberculosis*	*S. aureus*	*S. pyogenes*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*B. fragilis*	*B. thetaiotaomicron*
9a	96	>6.25	2	2	8	8	32	4−8	4−8
9b	100	3.13	6	n.d.[a]	n.d.	32	n.d.	4	4−8
agelasine D (**10**)	92	>6.25	1	2	8	8	16	16	8−16
rifampicin	>90	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
gentamycin	n.d.		0.03	1−2	>4	0.13	0.13	n.d.	n.d.
metronidazole	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	0.5	1

[a] No data obtained.

Table 3. Cytotoxic Activity of Compounds **9a**, **9b**, and Agelasine D (**10**), as Well as Three Clinically Used Drugs on the Cell Lines U-937 GTB, RPMI 8226/s, CEM/s, and ACHN

	IC_{50} (μM)			
compound	U-937 GTB (lymphoma)	RPMI 8226/s (myeloma)	CEM/s (leukemia)	ACHN (renal)
9a	1.03	0.2	2.33	8.01
9b	>80	>80	>80	>80
agelasine D (**10**)	5.59	3.7	4.68	24.97
doxorubicin	0.11	0.13	0.18	14.2
cisplatin	2.56	14.83	2.48	17.8
palitaxel	0.0059	0.007	0.007	31.5

drug-sensitive cell lines. The results are illustrated in Figure 2 and summarized in Table 3. Agelasine D (**10**) and the analogue **9a** exhibited potent activity against the myeloma cell line examined, as well as the other three cell lines. Both compounds showed a dose-dependent cytotoxicity and were cytotoxic in concentrations in the range of clinical drugs used today (Table 3). The activities were comparable with that reported for agelasine G against another lymphoma cell line (L1210; IC_{50} 3.1 μg/mL ≈ 4.8 μM).[10] Renal cell carcinomas (RCC) are quite resistant to chemotherapy;[16] hence it was especially intriguing to find that compound **9a** was more effective against the primary multidrug-resistant (MDR) cell line ACHN (renal adenocarcinoma cells)[17] than any of the anticancer drugs used as positive controls. The *tert*-butoxy derivative **9b** showed no activity at 80 μM against any of the cell lines examined. Due to its low toxicity, compound **9b** is an interesting antibacterial compound.

We have made significant improvements in our synthesis of (+)-agelasine D (**10**) and found that this marine natural product exhibits a variety of biological activities. Agelasine D and two close analogues display a broad spectrum of antibacterial activities including an effect on *M. tuberculosis*. The same compounds show profound inhibitory activity against several cancer cells including a multidrug-resistant cell line. It is plausible considering the cytotoxic and antibacterial activity of agelsine D that it can be involved in the host defense of sponges from *Agelas* spp. Further investigations with relevant assays are needed to clarify the role of agelasine D in the sponge. Our results demonstrate that agelasines are highly interesting antineoplastic agents, and the less cytotoxic analogues may have a potential as antibiotics. There is an urgent need for new antibacterial agents with novel mechanisms and chemical structures since resistance has been reported for virtually all existing antibacterial drugs.[18]

Experimental Section

General Experimental Procedures. Melting points are uncorrected. The ^{1}H NMR spectra were acquired on a Bruker Avance DPX 300 spectrometer at 300 MHz, and the ^{1}H decoupled ^{13}C NMR spectra were recorded at 75 MHz using the above-mentioned spectrometer. Mass spectra under electron impact conditions were recorded with a VG Prospec instrument at 70 eV ionizing voltage and are presented as *m*/*z* (% rel int). Electrospray MS were recorded with a Bruker Apex 47e FT-ICR mass spectrometer. Elemental analyses were performed by Ilse Beetz Mikroanalytisches Laboratorium, Kronach, Germany. DMA and *n*-butanol were distilled from BaO and stored over molecular sieves (3 Å), THF and diethyl ether were distilled from Na/benzophenone, and triethylamine was distilled from CaH_2. Acetic acid anhydride was refluxed over $CaCl_2$ for 5 days prior to distillation and kept over molecular sieves (3 Å). *O*-*tert*-Butylhydroxylamine hydrochloride was synthesized according to the literature[19] from *N*-(*tert*-butoxy)phthalimide.[20] Compounds **5**,[6] **6a**,[6] and **9a**[5] were prepared as described before, and antimycobacterial activities against *M. tuberculosis* $H_{37}Rv$ (ATCC27294) were determined as reported before.[6]

(+)-Manoyl Acetate (1). (+)-Manool (1.00 g, 3.44 mmol) and DMAP (0.33 g, 2.7 mmol) were dissolved in dry THF (30 mL) under N_2. Triethylamine (4.8 mL, 34 mmol) and Ac_2O (3.2 mL, 34 mmol) were added, and the mixture was refluxed for 40 h. The reaction mixture was cooled, diluted with diethyl ether (100 mL), washed with 2 M aqueous HCl (3 × 30 mL), saturated aqueous NH_4Cl (3 × 30 mL), and saturated aqueous NaCl (30 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOAc−hexane (1:70); yield 1.04 g (91%) as a colorless oil: $[\alpha]^{20}_D$ +24.8 (*c* 2.52, $CHCl_3$) (lit.[21] $[\alpha]_D$ +24.5; *c* 2.52, $CHCl_3$);

^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.97 (1H, dd, J = 17.5, 11.0 Hz), 5.16 (1H, dd, J = 17.5, 0.9 Hz), 5.13 (1H, dd, J = 11.0, 0.9 Hz), 4.92 (1H, d, J = 1.4 Hz), 4.50 (1H, d, J = 1.4 Hz), 2.39 (1H, ddd, J = 12.7, 4.2, 2.4 Hz), 2.05 (3H, s, CH_3), 2.00−0.95 (18H, m), 0.81 (3H, s, CH_3), 0.68 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3 (CO), 149.1, 142.4, 113.4, 106.8, 83.8, 57.6, 56.0, 42.6, 40.2, 39.7, 39.4, 38.7, 34.00, 33.97, 24.8, 23.9, 22.6, 22.1, 19.8, 17.8, 14.6.

(+)-Copalyl Acetate (2).[22] (+)-Manoyl acetate (**1**) (0.70 g, 2.1 mmol) and $PdCl_2(CH_3CN)_2$ (0.1 mmol, 28 mg) were dissolved in dry THF (20 mL) at 0 °C and stirred for 2 h. The mixture was evaporated and filtered through a small pad of silica gel eluting with EtOAc−hexane (1:20); yield 0.95 g (90%, *E*/*Z*: 92:8) as a colorless oil: $[\alpha]^{20}_D$ +30.1 (*c* 1.6, $CHCl_3$) (lit.[22] $[\alpha]_D$ +29.5; *c* 1.6, $CHCl_3$ for the *E*-isomer and $[\alpha]_D$ +8; *c* 9.2, $CHCl_3$ for the *Z*-isomer); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.32 (1H, m), 4.84 (1H, d, J = 1.3 Hz), 4.60 (2H, d, J = 7.1 Hz), 4.51 (1H, d, J = 1.3 Hz), 2.40 (1H, ddd, J = 12.6, 4.2, 2.4 Hz), 2.30−0.95 (21H, m), 0.88 (3H, s, CH_3), 0.81 (3H, s, CH_3), 0.69 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.5 (CO), 149.0, 143.5, 118.3, 106.6, 61.8, 56.6, 56.0, 42.6, 40.0, 39.5, 38.8, 38.7, 34.01, 33.97, 24.8, 22.1, 22.0, 21.5, 19.8, 16.9, 14.9; *anal.* C 79.42%, H 10.78%, calcd for $C_{22}H_{36}O_2$, C 79.46% H, 10.91%.

(+)-Copalol (3). A mixture of (+)-copalyl acetate (**2**) (814 mg, 2.25 mmol) and K_2CO_3 in methanol (3%) (1.2 g of K_2CO_3 in 38 g of MeOH) was stirred at ambient temperature overnight. The mixture was evaporated, diluted with water (50 mL), and extracted with diethyl ether (4 × 50 mL). The combined organic extracts were washed with saturated aqueous NaCl (25 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOH−EtOAc−hexane (1:5:50); yield 537 mg (76%, pure *E*-isomer) as a colorless oil: $[\alpha]^{20}_D$ +36.8 (*c* 1.14, $CHCl_3$) (lit.[9a] $[\alpha]_D$ +31.2; *c* 1.14, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.40 (1H, tq, J = 6.9, 1.2 Hz), 4.82 (1H, d, J = 1.4 Hz), 4.52 (1H, d, J = 1.1 Hz), 4.14 (2H, d, J = 6.9 Hz), 2.40 (1H, ddd, J = 12.7, 4.2, 2.4 Hz), 2.16 (1H, m), 1.99 (1H, dt, J = 12.9, 5.0 Hz), 1.90−0.95 (17H, m), 0.88 (3H, s, CH_3), 0.81 (3H, s, CH_3), 0.69 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.0, 141.0, 123.4, 106.6, 59.8, 56.7, 55.9, 42.6, 40.1, 39.5, 38.83, 38.76, 34.01, 33.98, 24.9, 22.2, 22.1, 19.8, 16.8, 14.9; EIMS *m*/*z* 290 $[M]^+$ (17), 275 (70), 272 (22), 257 (34), 205 (16), 137 (100); HREIMS *m*/*z* 290.2603 (calcd for $C_{20}H_{34}NO$, 290.2609).

Anticopalyl Bromide (4). (+)-Copalol (**3**) (870 mg, 3.00 mmol) was dissolved in dry diethyl ether (10 mL) under N_2 at 0 °C. PBr_3 (813 mg, 3.00 mmol) was added, and the mixture was stirred at 0 °C for 3 h. The mixture was diluted with diethyl ether (60 mL) and washed with 10% aqueous $NaHCO_3$ (20 mL). The aqueous phase was extracted with diethyl ether (20 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo; yield 841 mg (76%) of the crude compound as a pale yellow oil. Spectroscopic data were in agreement with those reported before.[5]

***N*^{6}-*tert*-Butoxy-9-methyl-9*H*-purin-6-amine (6b).** 6-Chloro-9-methyl-9*H*-purine (**5**) (0.60 g, 3.6 mmol) and *O*-*tert*-butylhydroxylamine hydrochloride (1.33 g, 10.6 mmol) were dissolved in dry *n*-butanol (18 mL) under N_2. Triethylamine (4.9 mL, 35 mmol) was added, and the mixture was refluxed overnight and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with EtOH−CH_2Cl_2 (1:14); yield 480 mg (61%) as colorless crystals, mp 235 °C. The compound existed as a 3:1 mixture of imino and amino tautomers in DMSO-d_6. ^{1}H NMR (DMSO-d_6, 300 MHz, data of major tautomer, as shown in Scheme 2) δ 10.80 (1H, br s, NH), 7.76 (1H, s, H-8), 7.56 (1H, d, J = 3.7 Hz, H-2), 3.63 (3H, s, NCH_3), 1.27 (9H, s, *t*-Bu); ^{13}C NMR (DMSO-d_6, 75 MHz data of major tautomer, as shown in Scheme 2) δ 145.0 (CH, C-2), 142.2 (C, C-4), 139.3 (CH, C-8), 141.6 (C, C-6), 119.4 (C, C-5), 77.6 (C, *t*-Bu), 30.3 (CH_3, NCH_3), 28.4 (CH_3, *t*-Bu); EIMS *m*/*z* 221 $[M]^+$ (2), 166 (9), 165 (100), 148 (8), 134 (7), 135 (69), 107 (6); HREIMS *m*/*z* 221.1271 (calcd for $C_{10}H_{15}N_5O$, 221.1276); *anal.* C 54.14%, H 6.67%, N 31.52%, calcd for $C_{10}H_{15}N_5O$, C 54.28%, H 6.83%, N 31.65%.

***N*^{6}-Benzyloxy-9-methyl-9*H*-purin-6-amine (6c).** A mixture of 6-chloro-9-methyl-9*H*-purine (**5**) (1.06 g, 6.29 mmol), *O*-benzylhydroxylamine hydrochloride (2.70 g, 16.9 mmol), and triethylamine (7.55 mL, 74.8 mmol) in dry *n*-butanol (50 mL) was stirred at reflux under N_2 for 16 h. The solvent was removed in vacuo, and the residue was stirred vigorously in MeOH (60 mL) at 50−60 °C until most of the residue was dissolved. The mixture was cooled to ca −50 °C [MeCN/CO_2(s)] for 10 min. The solid was filtered off, washed with diethyl ether (10 mL), and dried; yield 1.27 g (79%) as colorless crystals: mp 233−235 °C (lit.[23] 232−234 °C); ^{1}H NMR (DMSO-d_6, 300 MHz, data of major tautomer, as shown in Scheme 2) δ 11.22 (1H, br s, NH), 7.78 (1H, s, H-8), 7.60 (1H, d, J = 3.3 Hz, H-2), 7.54 (2H, m, Ph), 7.45−7.22 (3H, m, Ph), 5.02 (2H, s, CH_2), 3.63 (3H, s, CH_3); EIMS *m*/*z* 255 $[M]^+$ (36), 238 (19), 149 (19), 91 (100).

7-Benzyl-6-methoxyamino-9-methyl-7*H*-purinium (7a) and *N*^{6}-Benzyl-*N*^{6}-methoxy-9-methyl-9*H*-purin-6-amine (8a). *N*^{6}-Methoxy-9-methyl-9*H*-purin-6-amine (**6a**) (115 mg, 0.64 mmol) was dissolved in DMA (10 mL) at 50 °C under N_2 before benzyl bromide (0.12 mL, 0.96 mmol) was added. The mixture was stirred at 50 °C overnight and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2−MeOH saturated with NH_3 (9:1); yield **7a** 95 mg (55%). The fractions containing isomer **8a** were combined, evaporated, and purified by flash chromatography eluting with EtOH−EtOAc (1:18); yield **8a** 52 mg (30%).

7-Benzyl-6-methoxyamino-9-methyl-7*H*-purinium (7a): colorless crystals; mp 215−216 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (1H, s, H-8), 7.80 (1H, s, H-2), 7.46−7.43 (2H, m, Ph), 7.36−7.32 (3H, m, Ph), 5.64 (2H, s, CH_2), 3.83 (3H, s, OCH_3), 3.69 (3H, s, NCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.1 (CH, C-2), 147.8 (C, C-6), 145.0 (C, C-4), 134.1 (C, Ph), 129.3 (CH, C-8), 129.3, 129.2, 129.1 (each CH, Ph), 109.4 (C, C-5), 61.5 (CH_3, OCH_3), 53.0 (CH_2), 31.0 (CH_3, NCH_3); HRESIMS *m*/*z* 270.1345 (calcd for $C_{14}H_{15}N_5O$+H, 270.1349).

***N*^{6}-Benzyl-*N*^{6}-methoxy-9-methyl-9*H*-purin-6-amine (8a):**[24] colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.50 (1H, s, H-2), 7.81 (1H, s, H-8), 7.45−7.42 (2H, m, Ph), 7.32−7.24 (3H, m, Ph), 5.34 (2H, s, CH_2O), 3.83, 3.82 (each 3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.6 (C, C-6), 152.3 (CH, C-2), 151.7 (C, C-4), 141.0 (CH, C-8), 136.7 (C, Ph), 128.6, 128.3, 127.4 (each CH, Ph), 118.9 (C, C-5), 62.5 (CH_3, OCH_3), 53.8 (CH_2), 29.7 (CH_3, NCH_3); EIMS *m*/*z* 269 $[M]^+$ (12), 238 (100), 133 (12), 106 (23), 91 (52); HREIMS *m*/*z* 269.1275 (calcd for $C_{14}H_{15}N_5O$, 269.1276).

7-Benzyl-6-*tert*-butoxyamino-9-methyl-7*H*-purinium (7b) and *N*^{6}-Benzyl-*N*^{6}-*tert*-butoxy-9-methyl-9*H*-purin-6-amine (8b). The title compounds were prepared from *N*^{6}-*tert*-butoxy-9-methyl-9*H*-purin-6-amine (**6b**) (221 mg, 1.00 mmol) and benzyl bromide (0.18 mL, 1.5 mmol) as described for compounds **7a** and **8a** above. The products were isolated by flash chromatography on silica gel eluting with CH_2Cl_2−MeOH saturated with NH_3 (11:1); yield **7b** 268 mg (86%). The fractions containing isomer **8b** were combined, evaporated, and purified by flash chromatography eluting with EtOAc−hexane (1:1); yield **8b** 30 mg (9%).

7-Benzyl-6-*tert*-butoxyamino-9-methyl-7*H*-purinium (7b): pale yellow crystals; mp 210−211 °C (dec); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.61 (1H, s), 7.81 (1H, s), 7.52−7.49 (2H, m, Ph), 7.32−7.29 (3H, m, Ph), 5.75 (2H, s, CH_2), 3.80 (3H, s, CH_3), 1.21 (9H, s, *t*-Bu); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.1 (CH, C-2), 143.5 (C, C-4), 142.1 (C, C-6), 134.5 (C, Ph), 133.7 (CH, C-8), 129.1, 129.0, 128.6 (each CH, Ph), 110.6 (C, C-5), 77.8 (C, *t*-Bu), 52.6 (CH_2), 31.4 (CH_3, NCH_3), 27.6 (CH_3, *t*-Bu); HRESIMS *m*/*z* 312.1831 (calcd for $C_{17}H_{21}N_5O$+H, 312.1818).

***N*-Benzyl-*N*-*tert*-butoxy-9-methyl-9*H*-purin-6-amine (8b):** colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.53 (1H, s, H-2), 7.85 (1H, s, H-8), 7.35−7.28 (2H, m, Ph), 7.26−7.16 (3H, m, Ph), 6.07 (1H, br s, Ha in CH_2), 4.92 (1H, br s, Hb in CH_2), 3.85 (3H, s, NCH_3), 1.44 (9H, s, *t*-Bu); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.9 (C, C-6), 152.1 (CH, C-2), 151.6 (C, C-4), 140.6 (CH, C-8), 137.3 (C, Ph), 128.4, 128.1, 127.0 (each CH, Ph), 119.8 (C, C-5), 82.8 (C, *t*-Bu), 58.6 (CH_2), 29.7 (CH_3, NCH_3), 27.3 (CH_3, *t*-Bu); HRESIMS *m*/*z* 312.1806 (calcd for $C_{17}H_{21}N_5O$+H, 312.1818).

7-Benzyl-6-benzyloxyamino-9-methyl-7*H*-purinium (7c) and *N*^{6}-Benzyl-*N*^{6}-benzyloxy-9-methyl-9*H*-purin-6-amine (8c). The title compounds were prepared from *N*^{6}-benzyloxy-9-methyl-9*H*-purin-6-amine (**6c**) (255 mg, 1.00 mmol) and benzyl bromide (0.18 mL, 1.5 mmol) as described for compounds **7a** and **8a** above. The products were isolated by flash chromatography on silica gel eluting with CH_2Cl_2−MeOH saturated with NH_3 (12:1); yield **7c** 249 mg (72%). The fractions containing isomer **8c** were combined, evaporated, and purified by flash chromatography eluting with EtOAc−hexane (2:1); yield **8c** 41 mg (13%).

7-Benzyl-6-benzyloxyamino-9-methyl-7*H*-purinium (7c): pale yellow crystals; mp 183 °C (dec); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.69 (1H, br s, H-8), 7.84 (1H, s, H-2), 7.45−7.15 (10H, m, 2×Ph), 5.57 (2H, s, NCH_2), 5.07 (2H, s, OCH_2), 3.64 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 156.9 (CH, C-2), 147.6 (C, C-6), 145.1 (C, C-4),

139.7, 135.0 (each C, Ph), 131.9 (CH, C-8), 129.6, 129.5, 129.4, 128.0, 128.4, 127.7 (each CH, Ph), 109.7 (C, C-5), 76.1 (CH_2, OCH_2), 53.1 (CH_2, NCH_2), 31.4 (CH_3); HRESIMS *m*/*z* 346.1659 (calcd for $C_{20}H_{19}N_5O$+H, 346.1662).

***N*^{6}-Benzyl-*N*^{6}-benzyloxy-9-methyl-9*H*-purin-6-amine (8c):** colorless oil; ^{1}H NMR ($CDCl_3$, 200 MHz,) δ 8.57 (1H, s, H-2), 7.88 (1H, s, H-8), 7.55−7.25 (10H, m, 2×Ph), 5.28, 5.05 (each 2H, s, CH_2), 3.89 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.7 (C, C-6), 152.8 (CH, C-2), 152.3 (C, C-4), 141.5 (CH, C-8), 137.1, 136.1 (each C, Ph), 130.2, 129.4, 128.9, 128.8, 128.7, 127.9 (each CH, Ph), 119.7 (C, C-5), 77.7 (CH_2), 55.5 (CH_2), 30.2 (CH_3); EIMS *m*/*z* 345 $[M]^+$ (43), 329 (18), 328 (77), 223 (49), 91 (100); HREIMS *m*/*z* 345.1581 (calcd for $C_{20}H_{19}N_5O$, 345.1589).

(+)-*N*^{6}-*tert*-Butoxyagelasine D (9b). *N*^{6}-*tert*-Butoxy-9-methyl-9*H*-purin-6-amine (**6b**) (221 mg, 1.00 mmol) was dissolved in dry DMA (4 mL) under N_2 at 50 °C. Anticopalyl bromide (**4**) (493 mg, 1.40 mmol) in DMA (2 mL) was added. The mixture was stirred overnight at 50 °C and evaporated in vacuo, and the residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2−MeOH saturated with NH_3 (10:1); yield 445 mg (90%) as pale yellow crystals: mp 181 °C; $[\alpha]^{20}_D$ +25.1 (*c* 5.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.97 (1H, s, H-8), 7.84 (1H, s, H-2), 5.53 (1H, t, J = 7.2 Hz, CH), 5.10 (2H, d, J = 7.2 Hz, CH_2), 4.81 (1H, s), 4.69 (1H, s), 3.86 (3H, s, NCH_3), 2.38 (1H, ddd, J = 12.7, 3.5, 2.4 Hz), 2.22 (1H, m), 1.82 (3H, s, CH_3), 1.29 (9H, s, *t*-Bu), 2.00−0.90 (14H, m), 0.86 (3H, s, CH_3), 0.79 (3H, s, CH_3), 0.66 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.7 (CH, C-2), 148.8, 145.9 (each C, C═), 144.6 (C, C-4), 143.8 (C, C-6), 131.7 (CH, C-8), 116.6 (CH, CH═), 111.1 (C, C-5), 106.7 (CH_2, ═CH_2), 79.8 (C, *t*-Bu), 56.8, 56.0, 48.1 (CH_2), 42.1 (CH_2), 40.1, 39.5 (CH_2), 38.9 (CH_2), 38.7 (CH_2), 33.99, 33.96, 31.7 (CH_3, NCH_3), 28.1 (CH_3, *t*-Bu), 24.8 (CH_2), 22.1, 22.0 (CH_2), 19.7 (CH_2), 17.6, 14.9; HRESIMS *m*/*z* 494.3858 (calcd for $C_{30}H_{47}N_5O$+H, 494.3853).

(+)-Agelasine D (10). A mixture of (+)-*N*^{6}-*tert*-butoxyagelasine D (**9b**) (197 mg, 0.400 mmol), Zn (0.33 g, 5.0 mmol), and AcOH (0.4 mL) in MeOH (20 mL) and water (2 mL) was stirred vigorously at 75 °C for 40 h. The mixture was filtered and the solid washed with MeOH (20 mL). Saturated aqueous NaCl (10 mL) and water (10 mL) were added to the MeOH solution, and the mixture was stirred for 1 h at ambient temperature and evaporated in vacuo. The residue was transferred to a separatory funnel using 50% saturated aqueous NaCl (40 mL) and $CHCl_3$ (50 mL). The phases were separated, and the aqueous phase was extracted with $CHCl_3$ (4 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by flash chromatography on silica gel eluting with MeOH−CH_2Cl_2 (1:6); yield 90 mg (49%) as colorless crystals: mp 132−136 °C (lit.[5] 128−130 °C); $[\alpha]^{20}_D$ +11.0 (*c* 1.0, MeOH), (lit.[2a] $[\alpha]_D$ +10.4; *c* 1.1, MeOH). Spectroscopic data are as reported before.[5]

Determination of Antibacterial Activity against *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615), *E.* coli (ATCC 25922), *E. faecalis*, and *P. aeruginosa* (ATCC27853). The minimum inhibitory concentrations (MIC) of the agelasines were determined using a standard broth microdilution technique as previously described.[25] Overnight cultures of bacteria were grown to exponential growth phase and diluted in growth medium to give a final inoculum of 1 × 10^6 CFU/mL. Bacteria (60 μL) and serial dilutions of agelasines in doubley distilled water (60 μL) were added to a 96-well microtiter plate (Nunc, Roskilde, Denmark) and incubated at 37 °C overnight. The MIC was determined as the lowest concentration where no visible growth occurred. Bactopeptone water (2%) (Difco, Detroit) was used as growth medium for *S. aureus*, *E. coli*, and *P. aeruginosa* and brain heart infusion broth (Oxoid Ltd, Basingstroke, Hampshire, UK) for *E. faecalis* and *S. pyogenes*. Gentamycin (Invitrogen Ltd, Paisley, UK) was used as internal control in all experiments.

Determination of Antibacterial Activity against *B. fragilis* (DSM 2151) and *B. thetaiotaomicron* (DSM 2255). Reference strains of *Bacteroides* spp. were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) GmbH, Braunschweig, Germany. The *Bacteroides* strains were maintained as thick suspensions in sterile deionized water at −80 °C. Prior to experiment strains were grown on blood agar plates at 35 °C for 24 h. Anaerobosis was created using anaerobe jars and bags (Oxoid Ltd, Basingstoke, Hampshire, UK). Reagent grade metronidazole (control) and compounds **9a**, **9b**, and **10** were prepared as stock solutions of 5120 μg/mL according to the recommendations of the National Committee for Clinical Laboratory Standards[26] using dimethyl sulfoxide and sterile deionized water as the solvent and dilutant, respectively. Stock solutions were stored in polyethylene vials at −80 °C until the day of use. After the antimicrobials were thawed, serial 2-fold dilutions were prepared in reduced Wilkins-Chalgren broth (Oxoid). Working concentrations ranging from 1.25 to 320 μg/mL were prepared to obtain final antibiotic concentrations of 0.125 to 32 μg/mL after dilution in broth. A direct suspension of colonies was prepared in 10 mL of Wilkins-Chalgren broth. The turbidity of the suspension was adjusted spectrophotometrically to match a 0.5 McFarland standard. This suspension was further diluted 1:10 with broth to obtain the inoculum having a concentration of about 1.5 × 10^7 CFU/mL. Susceptibility testing was performed using the broth microdilution technique.[26] Dilutions (100 μL) were added in triplicate to the wells of a microtiter plate. To each well was added 10 μL of the inoculum. Broth alone, broth amended with cells, and broth amended with antimicrobial without cells were used as controls. After inoculation, plates were lidded and incubated anaerobically at 35 °C for 48 h. The MIC value was read as the lowest concentration of antimicrobial agent showing no visible growth. Tests were repeated at least once with fresh samples and a fresh inoculum.

Determination of Cytotoxic Activities. A fluorometric microculture cytotoxity assay (FMCA) was used to determine cytotoxicity of **9a**, **9b**, and **10**.[27] Compounds were dissolved in 10% DMSO and added to 96-well microtiter plates in the following 10 concentrations: 80, 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 μM. Each concentration was applied in triplicate. Solvent controls (10% DMSO), blank controls (cell-growth medium), positive controls (Triton X-100), and negative controls (phosphate-buffered saline solution, PBS) were prepared for each microtiter plate. The tumor cells, suspended in cell-growth media, were dispensed in the prepared microtiter plates. After 72 h of incubation, the cells were washed with PBS, and FDA was added. The plates were reincubated for 40 min, and the generated fluorescence was measured. The fluorescence is proportional to the number of living cells, which reflects cell survival, and provides a quantifiable survival index (SI).

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