Full Paper

Synthesis, Antimicrobial and Antineoplastic Activities for Agelasine and Agelasimine Analogs with a β-Cyclocitral Derived Substituent

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Agelasines and agelasimines are antimicrobial and cytotoxic purine derivatives isolated from marine sponges (*Agelas sp.*). We have synthesized structurally simplified analogs of these natural products starting from β-cyclocitral. The novel compounds were found to be strong inhibitors of a wide variety of pathogenic microorganisms (incl. *Mycobacterium tuberculosis*) as well as cancer cell lines. The biological activities were generally in the same range as those previously found for the structurally more complex agelasines and agelasimines isolated in small amounts from natural sources. We also report for the first time that agelasine and agelasimine analogs inhibit growth of protozoa (*Acanthamoeba castellanii* and *Acanthamoeba polyphaga*). *Acanthamoeba* keratitis is an increasingly common and severe corneal infection, closely associated with contact lens wear.

Keywords: Agelasimine / Agelasine / Anti-cancer activity / Antimicrobial activity / β-Cyclocitral

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Introduction

Agelasines [1 – 7] and agelasimines [8, 9] are antimicrobial and cytotoxic purine derivatives isolated from marine sponges (*Agelas sp.*). In both classes of compounds, there is a diterpenoid substituent in the purine 7-position. Some examples of agelasines and agelasimines are shown in Fig. 1.

We have completed the first synthesis of agelasine E [10], and we recently reported an efficient synthesis of agelasine D from manool [11, 12]. High activity against cancer cell lines and bacteria were found for agelasine D and synthetic intermediates [12]. Since agelasines and agelasimines are found in only minute amounts in nature and total syntheses of these compounds are relatively complex, it would be highly interesting if analogs, more easily synthetically available, but still with potent bioactivities, could be developed. Herein, we report synthesis of compounds closely related to agelasine E and F, with a terpenoid side chain easily available from β-cyclocitral, as well as agelasimine analogs carrying the same terpenoid substituent. Activities against various pathogenic microorganisms and cancer cell lines have been determined.

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Abbreviation: minimum trophocidal concentrations (MTC)

Results and discussion

β-Cyclocitral **1** was readily reduced to the corresponding alcohol **2** and further converted to the bromide **3** according to literature procedures [13] (Scheme 1). The alcohol **9** has been formed in moderate yields, when the bromide

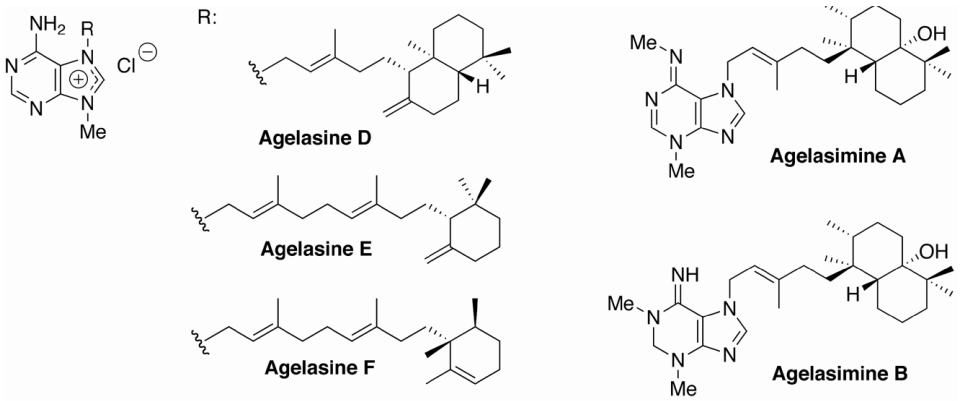
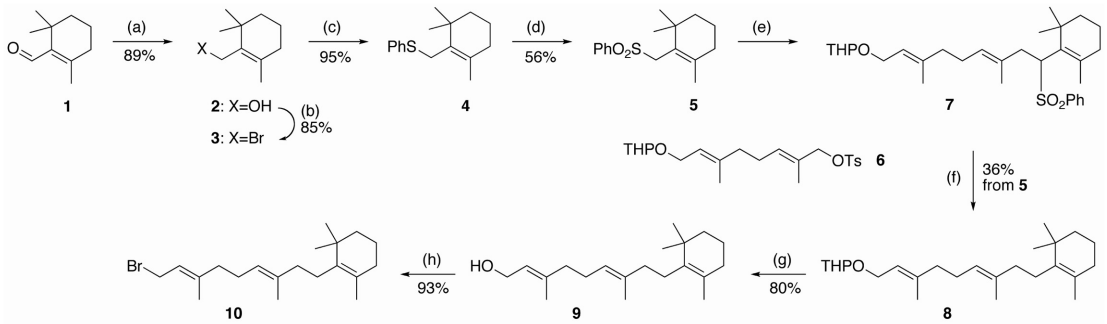


Figure 1. Structures of some agelasines and agelasimines.



Reaction conditions: (a) $NaBH_4$, EtOH, *i*-PrOH; (b) PBr_3, Et_2O, hexane, −30°C; (c) PhSSPh, Bu_3P, pyridine; (d) oxone, MeOH, H_2O; (e) 1. *n*-BuLi, 2. DMPU, comp. **6**, THF, 0°C; (f) NaHg, Na_2HPO_4, MeOH; (g) PPTS, EtOH, 55°C; (h) PBr_3, Et_2O.

Scheme 1. Synthesis route of compounds **1**–**10**.

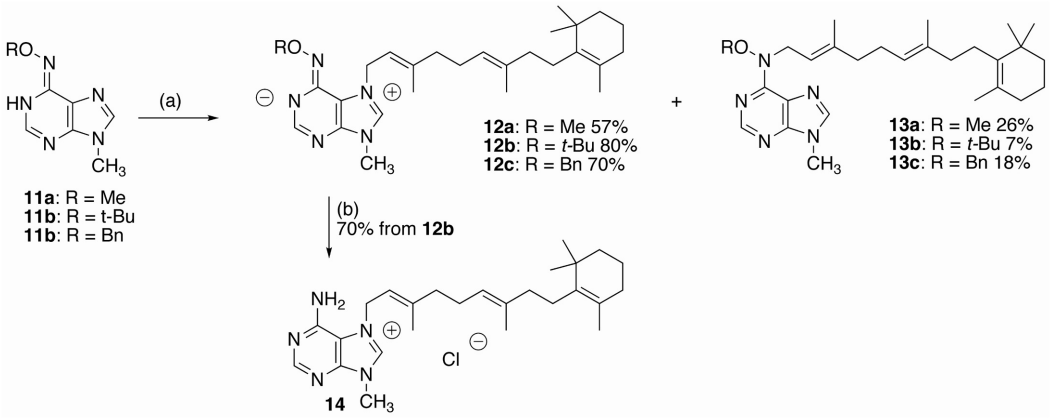
3 was converted to the corresponding Grignard reagent and reacted in large excess with a suitable geraniol derivative [14]. However, we found conversion of the bromide to the Grignard reagent sluggish, and, instead, we chose to couple the cyclocitral and geraniol derived monoterpenes by reacting the lithiated sulfone **5** with the geraniol derived tosylate **6**, essentially the same strategy as we applied in the synthesis of the agelasine E side chain [15]. Synthesis of the alkylating agent **10** from β-cyclocitral **1** is shown in Scheme 1. Sulfone **5** has previously been synthesized by a non-selective cyclization of geranyl phenyl sulfone [16].

The agelasine E and F analog **14** was available by alkylation of adenine derivatives **11** with the allylic bromide **10** followed by reductive removal of the alkoxy-directing group (Scheme 2). As also observed in the agelasine D synthesis [12], the regioselective outcome in the *N*-alkylation step is highly dependant on the size of the N^6-alkoxy group in compounds **11**. *tert*-Butoxy derivative **12b** was formed with high regioselectivity whereas the selectivity in the alkylation of the N^6-methoxyadenine **11a** was more modest.

Also agelasimine analogs with the cyclocitral derived side chain were synthesized (Scheme 3). 3-Methyladenine **15** [17] was alkylated with complete selectivity on *N*-7 and the product **16** could be methylated at N^6 to give the agelasimine A analog **17** or reduced to compound **18** followed by *N*-1 methylation to give the agelasimine B analog **19**. The modest yields in the agelasimine directed reactions are probably a result of lower chemical stability of agelasimine derivatives **16**–**19** compared to the related agelasine derivatives **12** and **14**. Especially compound **19** appeared to decompose readily and we chose not to include this agelasimine analog in the study of bioactivities (see below).

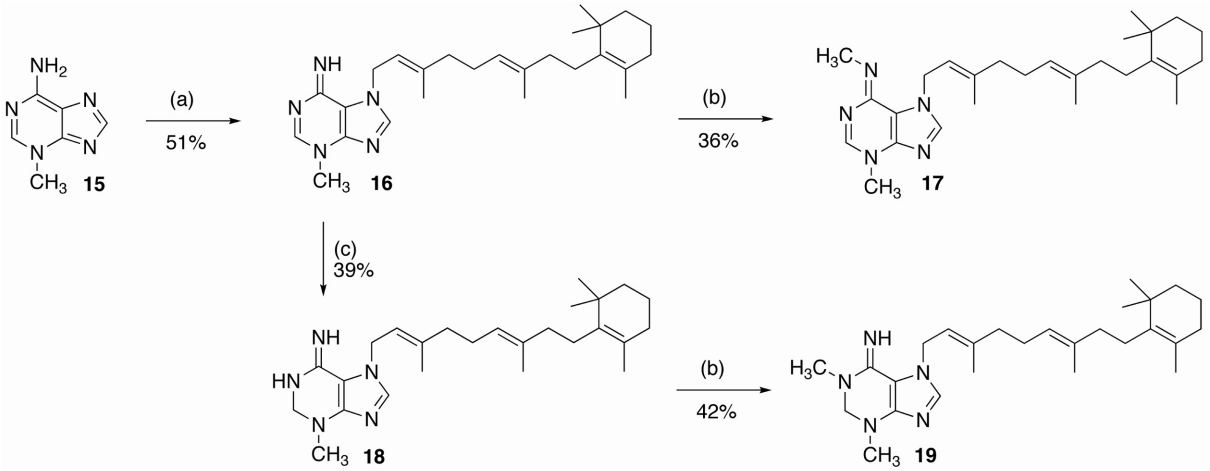
Antimicrobial activities for agelasine analogs **12** and **14** and agelasimine analogs **16**–**18** were examined and the results are presented in Table 1. In addition to *Staphylococcus aureus* and *Escherichia coli*, also a mycobacterium (*Mycobacterium tuberculosis*) and pathogenic protozoa (*Acanthamoeba castellanii* and *Acanthamoeba polyphaga*) were included in the study.

Identification of compounds, which inhibit mycobacterial growth, is very important. There has been no



Reaction conditions: (a) Comp. **10**, DMA, 50°C; (b) Zn, AcOH, MeOH, H_2O, 75°C.

Scheme 2. Synthesis route of compounds **11** – **14**.



Reaction conditions: (a) Comp. **10**, DMA, 50°; (b) MeI, DMA; (c) $NaBH_4$, MeOH, H_2O.

Scheme 3. Synthesis route of compounds **15** – **19**.

Table 1. Antimicrobial activity of agelasine and agelasimine analogs against *Staphylococcus aureus, Escherichia coli*, *Mycobacterium tuberculosis*, *Acanthamoeba castellanii,* and *Acanthamoeba polyphaga.*

Comp.	MIC *S. aureus* (μg/mL)[a]	MIC *E. coli* (μg/mL)[b]	% Inhib. *M. tuberculosis* at 6.25 μg/mL[c]	MTC *A. castellani* (μg/mL)[d]	MTC *A. polyphaga* (μg/mL)[d]
12a	4	16	100	64	64
12b	4	>32	100	64 – >64	64 –>>64
12c	6	>32	46	>64	>64
14	16	>32	95	>64	>64
16	8	16	99	32	32
17	4	32	100	32	32
18	8	>32	100	64 – >64	64 –>>64

[a] MIC gentamycin 0.1 μg/mL.
[b] MIC gentamycin 0.5 μg/mL.
[c] rifampin:% Inhib. at 6.25 μg/mL >90. MIC 0.2 μg/mL.
[d] MTC benzalkonium chloride 64 μg/mL.

launch of new drugs to treat tuberculosis for approximately 40 years, even though the disease claims ca. two million lives every year, and infections with multi-drug resistant strains are an increasing problem [18, 19].

Acanthamoeba species are predominantly free-living protozoa found ubiquitously throughout the environment. They are characterized by a feeding and replicate trophozoite and dormant cyst stage [20]. They are recognized as the cause of a keratitis and granulomatous encephalitis in humans [21, 22]. *Acanthamoeba* keratitis is an increasingly common and severe corneal infection. It is closely associated with contact lens wear (approximately 95% of reported cases) and can affect immunocompetent individuals [23–27]. Infection results from contamination of lens care products, notably the lens storage case, from which the organism adheres to the contact lens and is inoculated onto the cornea [20, 28]. Present therapeutic regimens for *Acanthamoeba* keratitis rely on topical applications of antimicrobials including a combination of propamide isethionate and neomycin or chlorohexidine. The need for these drugs to be applied every 15–60 min. for a period of weeks makes treatment arduous. Corneal transplantation is often necessary due to the extensive damage caused by the parasites [29]. Since agelasines and analogs generally displays a broad spectrum of antimicrobial activities, it was thus interesting to see if agelasines or their derivatives showed activity *Acanthamoeba sp.*, and if so to determine if such activity was achieved at therapeutically interesting concentrations.

As observed for other agelasine analogs before [10, 12], also the compounds examined in the current study displayed profound antibacterial activities including activity against *M. tuberculosis* (Table 1). Also interesting inhibitory activities against *Acanthamoeba sp.*, were found. No generally accepted, standardized method for testing of the efficacy of antimicrobials against *Acanthamoeba* exists [30]. In the present study, the antimicrobial efficacy of agelasines and their derivatives was investigated using a microtiter-plate based assay, in which *Acanthamoeba* trophozoites are mixed with doubling-dilutions of the active agent and heat-killed *Escherichia coli* as carbon and energy source. The number of trophozoites surviving incubation was estimated based on a staining procedure using tryptan blue. The inoculum prepared as described in section 3 (Experimental), contained >99% of trophozoites excluding tryptan blue, and these were taken to be viable cells. Table 1 provides the range of minimum trophocidal concentrations (MTC) obtained based on two test runs and using the criteria outline in the experimental section. Although identical MTC values were recorded for both species, examination of samples at lower agent concentrations indicated, based on the number of viable trophozoites, that *A. polyphaga* was the most resistant of the strains. This is in keeping with some previous observations [31]. Based on the same criteria, it can also be noted that compound **16** was the most effective agent. MTC values for benzalkonium chloride are greater than that (12 μg/mL) recorded previously for a different strain of *A. castellanii* grown as broth culture [32]. However, the present analysis showed that blue (non-viable) trophozoites dominated when the concentration of benzalkonium chloride exceeded 8 μg/mL. Factors that could account for the differences include the differing methods of cultivation, and the stringency of the criteria for non-viability.

Table 2. Cytotoxic activity of agelasine and agelasimine analogs on the cell lines U-937 GTB (lymphoma), RPMI 8226/s (myeloma), CEM/s (leukemia), and ACHN (renal).

Comp.	IC_{50}			
	U-937 GTB (μM)[a]	RPMI 8226/s (μM)[b]	CEM/s (μM)[c]	ACHN (μM)[d]
12a	3.46	6.34	8.64	16.4
12b	3.01	4.33	6.01	7.76
12c	6.07	7.60	9.79	11.5
14	6.28	10.2	10.4	47.0
16	3.10	2.76	2.91	7.23
17	5.41	4.42	5.00	10.5
18	7.21	4.77	5.61	10.8

[a] IC_{50} doxorubicin 0.11 μM, IC_{50} cisplatin 2.56 μM, IC_{50} palitaxel 0.0059 μM.

[b] IC_{50} doxorubicin 0.13 μM, IC_{50} cisplatin 14.83 μM, IC_{50} palitaxel 0.007 μM.

[c] IC_{50} doxorubicin 0.18 μM, IC_{50} cisplatin 2.48 μM, IC_{50} palitaxel 0.007 μM.

[d] IC_{50} doxorubicin 14 2 μM, IC_{50} cisplatin 17.8 μM, IC_{50} palitaxel 31.5 μM.

Agelasine D and close analogs were previously found to exhibit inhibitory activity against several cancer cell lines, including the drug resistant renal cancer cell line (ACHN) [12], and hence agelasine analogs **12** and **14** and agelasimine analogs **16–18** were examined potential anti-cancer compounds (Table 2). The agelasine and agelasimine analogs generally exhibited profound cytotoxic activity and there were only minor differences found between the compounds. Agelasine **14**, however, was significantly less active against the ACHN cells than the other compounds examined.

The novel agelasine and agelasimine analogs described herein were found to be excellent inhibitors of a wide variety of pathogenic microorganisms (incl. *Mycobacterium tuberculosis* and *Acanthamoeba sp.*) and cancer cell lines. The biological activities were generally in the same range as those previously found for the structurally more complex agelasines and agelasimines isolated in small amounts from natural sources or synthesized by tedious

routes [1 – 10, 12]. We have demonstrated that analogs of these natural products containing a synthetically less demanding terpenoid side chain, may still exhibit the same potent bioactivities. Further studies towards more selective agelasine and agelasimine analogs are in progress.

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Experimental

The ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker Avance DPX 300 instrument or at 200 MHz with a Bruker Avance DPX 200 instrument (Bruker, Rheinstetten, Germany). The 1H decoupled ^{13}C-NMR spectra were recorded at 75 or 50 MHz using instruments mentioned above. Mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing voltage with a VG Prospec instrument (Micromass, Manchester, UK), and are presented as *m*/*z* (% rel. int.). Electrospray MS spectra were recorded with a Bruker Apex 47e FT-ICR mass spectrometer (Bruker). Elemental analyses were performed by Ilse Beetz Mikroanalytisches Laboratorium, Kronach, Germany. Melting points are uncorrected. DMA was distilled from BaO and stored over 4 -Å molsieve; pyridine and DMPU were distilled from CaH_2, and THF and diethyl ether from Na/benzophenone.

The following compounds were prepared according to literature procedures: 2,6,6-Trimethyl-1-cyclohexene-1-methanol **2** [13], 2-(bromomethyl)-1,3,3-trimethylcyclohexene **3** [13], (2*E*,6*E*)-3,6-dimethyl-8-(tetrahydro-2*H*-pyran-2-yloxy)octa-2,6-dienyl-4-methylbenzenesulfonate **6** [15], N^6-methoxy-9-methyl-9*H*-purin-6-amine **11a** [10], N^6-*tert*-butoxy-9-methyl-9*H*-purin-6-amine **11b** [12], N^6-benzyloxy-9-methyl-9*H*-purin-6-amine **11c** [12] and 3-methyladenine **15** [17]. Antimicrobial activities against *S. aureus* and *E. coli* and *M. tuberculosis* H_{37}Rv (ATCC 27294) were determined as reported before [12, 33]. Activity against cancer cell lines (U-937 GTB, RPMI 8226/s, CEM/s and ACHN) investigated using a fluorometric microculture cytotoxicity assay (FMCA) [34] as described before [12].

Determination of activity against *A. castellanii* (CCAP 1501/A) and *A. polyphaga* (CCAP 1501/18) Strains used in the study were *Escherichia coli* (ATCC 25922), *Acanthamoeba castellanii* (CCAP 1501/A) and *Acanthamoeba polyphaga* (CCAP 1501/18). Protozoal cultures were obtained from Culture Collection of Algae and Protozoa (CCAP, Dunstaffnage Marine Laboratory, Argyll in axenic form in broth medium. All chemicals were of at least analytical grade. Tryptan Blue (0.4%; catalog number T8154), and benzalkonium chloride (ultrapure; catalog number B-6295) used as standard for antimicrobial testing were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Chemistry

*{[(2,6,6-Trimethyl-1-cyclohexen-1-yl)methyl]thio}benzene **4***

Tributylphosphine (2.60 mL, 10.7 mmol) was added to a stirring mixture of 2,6,6-trimethyl-1-cyclohexene-1-methanol **2** (596 mg, 3.87 mmol) and diphenyldisulfide (2.48 g, 11.4 mmol) in dry pyridine (1.6 mL) at ambient temperature under N_2-atm. The resulting mixture was stirred for 13 h, diluted with EtOAc (35 mL), washed with 10% aq. HCl (9 mL), 10% aq. NaOH (9 mL) and brine (9 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The product was purified by flash chromatography on silica gel eluting with hexane-acetone (100 : 1); yield 900 mg (95%), colorless liquid. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.07 (s, 6H), 1.43 – 1.47 (m, 2H), 1.48 – 1.60 (m, 2H), 1.73 (s, 3H), 1.98 (t, *J* = 6.3 Hz, 2H), 3.60 (s, 2H), 7.12 – 7.29 (m, 5H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 19.2, 20.2, 28.6 (2 × CH_3), 32.6, 32.9, 34.7, 39.4, 125.3, 128.2 (CH in Ph), 128.6 (CH in Ph), 131.9, 133.6, 139.1; MS EI *m*/*z* (rel.%) 246 (26) [M^+], 137 (100), 136 (52), 121 (25), 109 (23), 95 (57), 93 (15). The spectral data are in good agreement with those reported before [35].

*{[(2,6,6-Trimethyl-1-cyclohexen-1-yl)methyl]sulfonyl}benzene **5***

A solution of oxone (17.0 g, 27.7 mmol) in water (75 mL) was added to a stirring solution of the sulfide **4** (800 mg, 3.24 mmol) in MeOH (75 mL) at 0°C, and the resulting mixture was stirred for 19 h at ambient temperature. Diethyl ether (400 mL) was added and the mixture was washed with water (200 mL) and brine (100 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The crude product was recrystallized from MeOH (5 mL); yield 2.86 g (56%), mp 89 – 91°C, colorless crystals. ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.00 (s, 6H), 1.42 – 1.46 (m, 2H), 1.57 – 1.61 (m, 2H), 1.62 (s, 3H), 2.01 (t, *J* = 6.4 Hz, 2H), 3.92 (s, 2H), 7.50 – 7.59 (m, 3H), 7.86-7.91 (m, 2H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 18.8, 21.7, 28.7 (2 × CH_3), 33.2, 34.3, 39.3, 57.4, 125.7, 127.7 (2 × CH in Ph), 129.0 (2 × CH in Ph) 133.2, 139.1, 141.4; MS EI *m*/*z* (rel.%) 278 (2) [M^+], 138 (12), 137 (100), 121 (8), 95 (37), 81 (24).

*(2E,6E)-9-Benzenesulfonyl-9-(2,6,6-trimethylcyclohex-1-enyl)-3,7-dimethyl-1-(tetrahydro-2H-pyranyloxy)-2,6-nonadiene **7***

n-Butyllithium (6.4 mL, 1.6 M, 10.2 mmol) was added dropwise to a stirring solution of sulfone **5** (1.04 g, 5.10 mmol) in dry THF (30 mL) at 0°C under N_2-atm. and the resulting mixture was stirred at 0°C for 30 min. A solution of crude tosylate **6** (2.08 g, 5.10 mmol) and DMPU (7.6 mL, 64 mmol) in dry THF (10 mL) was added. The reaction was stirred for 20 h while reaching ambient temperature. Diethyl ether (130 mL) was added and the mixture was washed with sat. aq. NH_4Cl (65 mL), water (3 × 65 mL) and brine (65 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The product was partially purified by flash chromatography on silica gel eluting with hexane/acetone (14 : 1), yield 1.631 g (containing 20 – 25% starting material **5**), pale yellow oil. This material was used directly in the next step.

(2E,6E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)-1-(tetrahydro-2H-pyranyloxy)-2,6-nonadiene ***8***

To a mixture of sulfone **7** (1.23 g, cont. 20 – 25% of comp. **5**) and Na_2HPO_4 (1.65 g, 12.0 mmol) in MeOH (30 mL) was added 10% NaHg (3.82 g, 17.0 mmol Na) and the resulting mixture was stirred at ambient temperature for 2 h. Water (70 mL) and diethyl ether (140 mL) was added, the mixture was decanted and the phases separated. The ethereal layer was washed with sat. aq. NH_4Cl (70 mL) and brine (70 mL), dried ($MgSO_4$), and evaporated *in vacuo*. The product was purified by flash chromatography on silica gel containing 20% (w/w) $AgNO_3$ eluting with EtOAc/hexane (1 : 20); yield 36% from compound **5**, colorless oil. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.97 (s, 6H, 2 × CH_3), 1.37 – 1.47 (m, 2H, CH_2), 1.51 – 1.61 (m, 6H, CH_2), 1.58 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.66 – 1.85 (m, 2H, CH_2 in THP), 1.88 (t, *J* = 6.2 Hz, 2H, CH_2), 1.89 – 2.11 (m, 8H, 4 × CH_2), 3.47 – 3.50 (m, 1H, H-6_a in THP), 3.83 – 3.87 (m, 1H, H-6_b in THP), 4.00 (dd, *J* = 11.9 and 7.4 Hz, 1H, H_a in OCH_2), 4.21 (dd, *J* = 11.9 and 6.4 Hz, 1H, H_b in OCH_2), 4.61 (t, *J* = 2.8 Hz, 1H, H-2 in THP), 5.11 (t, *J* = 5.7 Hz, 1H, CH=), 5.34 (t, *J* = 6.4 Hz, 1H, CH=); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 16.0 (CH_3), 16.4 (CH_3), 19.5 (CH_2), 19.6 (CH_2 in THP), 19.8 (CH_3), 25.5 (CH_2 in THP), 26.3 (CH_2), 27.9 (CH_2), 28.6 (2 × CH_3), 30.7 (CH_2 in THP), 32.7 (CH_2), 35.0 (C-6 in cyclohexene), 39.6 (CH_2), 39.9 (CH_2), 40.3 (CH_2), 62.2 (C-6 in THP), 63.6 (OCH_2), 97.8 (C-2 in THP), 120.6 (CH=), 123.3 (CH=), 126.9 (C=), 136.3 (C=), 137.2 (C=), 140.2 (C=); MS EI *m/z* (rel.%) 374 (2) [M^+], 273 (21), 204 (22), 137 (100), 136 (28), 121 (17), 95 (35), 85 (94), 81 (26); HRMS (EI) Found 374.31898, $C_{25}H_{42}O_2$ requires 374.3185; Anal. Calcd.: Found: C, 79.66; H, 11.52. Calc. for $C_{25}H_{42}O_2$: C, 80.16; H, 11.30%.

(2E,6E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-ol ***9***

A mixture of the THP-ether **8** (415 mg, 1.11 mmol) and pyridinium *p*-toluenesulfonate (66 mg, 0.26 mmol) in EtOH (13 mL) was stirred at 55°C under N_2-atm. for 13 h, before the mixture was evaporated *in vacuo* and the residue was purified by flash chromatography on silica gel eluting with hexane/acetone (15 : 1); yield 256 mg (80%), pale yellow oil. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.97 (s, 6H, 2 × CH_3), 1.37-1.41 (m, 2H, CH_2), 1.51 – 1.55 (m, 2H, CH_2), 1.57 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.67 (s, 3H, CH_3), 1.88 (t, *J* = 6.2 Hz, 2H, CH_2), 1.90 – 2.11 (m, 8H, 4 × CH_2), 4.14 (d, *J* = 7.0 Hz, 2H, OCH_2), 5.11 (t, *J* = 5.8 Hz, 1H, CH=), 5.41 (td, *J* = 7.0 and 1.2 Hz, 1H, CH=); ^{13}C-NMR ($CDCl_3$, 75 MHz) d 16.0 (CH_3), 16.3 (CH_3), 19.6 (CH_2), 19.8 (CH_3), 26.3 (CH_2), 27.9 (CH_2), 28.6 (2 × CH_3), 32.8 (CH_2), 35.0 (C-6 in cyclohexene), 39.6 (CH_2), 39.9 (CH_2), 40.3 (CH_2), 59.4 (OCH_2), 123.1 (CH=), 123.3 (CH=), 126.9 (C=), 136.4 (C=), 137.1 (C=), 139.9 (C=); MS EI *m/z* (rel.%) 290 (5) [M^+], 138 (20), 137 (100), 136 (23), 121 (12), 95 (36), 93 (9), 81 (24); HRMS (EI) Found 290.2613, $C_{20}H_{34}O$ requires 290.2610; Anal. Calcd.: Found: C, 82.81; H, 11.66. Calc. for $C_{20}H_{34}O$: C, 82.69; H, 11.80%.

(2E,6E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl bromide ***10***

The allylic alcohol **9** (200 mg, 0.69 mmol) was dissolved in dry diethyl ether (2.5 mL) under N_2-atm. at 0°C. PBr_3 (0.065 mL, 0.70 mmol) was added and the mixture was stirred 0°C for 3 h, diluted with diethyl ether (15 mL) and washed with 10% aq. $NaHCO_3$ (5 mL). The aqueous phase was extracted with diethyl ether (5 mL) and the combined organic extracts were dried ($MgSO_4$), and evaporated *in vacuo*; yield 228 mg (93%), pale yellow oil which was used in alkylation reactions without further purification. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.97 (s, 6H), 1.37 – 1.41 (m, 2H), 1.48 – 1.56 (m, 2H), 1.58 (s, 3H), 1.62 (d, *J* = 1.5 Hz, 3H), 1.71 (d, *J* = 1.4 Hz, 3H), 1.88 (t, *J* = 5.9 Hz, 2H), 1.91-2.07 (m, 8H), 4.01 (d, *J* = 8.4 Hz, 2H), 5.05 – 5.09 (m, 1H), 5.52 (td, *J* = 8.5 and 1.2 Hz, 1H).

7-[(2′E,6′E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl]-6-methoxyamino-9-methyl-7H-purinium ***12a*** *and* ***N^6-[(2′E,6′E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl]-N^6-methoxy-9-methyl-9H-purin-6-amine 13a***

A mixture of *N*^{6}-metoxy-9-methyl-9*H*-purin-6-amine **11a** (42 mg, 0.23 mmol) and allylic bromide **10** (98 mg, 0.28 mmol) in dry DMA (2 mL) was stirred at 50°C under N_2-atm. for 21 h and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH sat. with NH_3 (9 : 1); yield **12a** 60 mg (57%). The fractions containing isomer **13a** were combined, evaporated and purified by flash chromatography eluting with EtOH-EtOAc (1 : 15); yield **13a** 27 mg (26%).

12a

Mp 178 – 180°C, pale yellow crystals. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.94 (s, 6H, 2 × CH_3), 1.35 – 1.39 (m, 2H, CH_2), 1.48-1.52 (m, 2H, CH_2), 1.53 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.86 (t, *J* = 6.1Hz, CH_2), 1.91 – 2.11 (m, 8H, 4 × CH_2), 3.72 (s, 3H, NCH_3), 3.79 (s, 3H, OCH_3), 5.03-5.05 (m, 3H, NCH_2 and CH=), 5.41 (t, *J* = 6.8 Hz, 1H, CH=), 7.77 (s, 1H, H-2), 7.89 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 16.0 (CH_3), 16.8 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.0 (CH_2), 27.8 (CH_2), 28.6 (2 × CH_3), 31.0 (NCH_3), 32.7 (CH_2), 34.9 (C-6 in cyclohexene), 39.5 (CH_2), 39.8 (CH_2), 40.3 (CH_2), 47.6 (NCH_2), 61.4 (OCH_3), 109.5 (C-5), 115.9 (CH=), 122.6 (CH=), 127.0 (C=), 128.9 (C-8), 136.9 (2 × C=), 145.1 (C-4), 145.6 (C=), 147.7 (C-6), 157.0 (C-2); HRMS (ESI) Found 452.3372, $C_{27}H_{41}N_5O$+H requires 452.3383.

13a

Colorless oil. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.95 (s, 6H, 2 × CH_3), 1.35 – 1.39 (m, 2H, CH_2), 1.49 – 1.57 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.89 (t, *J* = 6.2 Hz, 2H, CH_2), 1.91 – 2.10 (m, 8H, 4 ' CH_2), 3.79 (s, 3H, NCH_3), 3.81 (s, 3H, OCH_3), 4.66 (d, *J* = 6.9 Hz, 2H, NCH_2), 5.02 (d, *J* = 6.7 Hz, 1H, CH=), 5.43 (t, *J* = 6.3 Hz, 1H, CH=), 7.80 (s, 1H, H-8), 8.47 (s, 1H, H-2); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 16.0 (CH_3), 16.6 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.3 (CH_2), 27.9 (CH_2), 28.6 (2 × CH_3), 29.8 (NCH_3), 32.7 (CH_2), 35.0 (C-6 in cyclohexene), 39.7 (CH_2), 39.8 (CH_2), 40.3 (CH_2), 48.3 (NCH_2), 61.6 (OCH_3), 118.4 (CH=), 119.1 (C-5), 123.3 (CH=), 126.9 (C=), 136.3 (C=), 137.1 (C=), 140.6 (C=), 141.0 (C-8), 151.7 (C-4), 152.2 (C-2), 155.5 (C-6); MS EI *m/z* (rel.%) 451 (5) [M^+], 246 (57), 217 (23), 216 (100), 179 (32), 162 (23), 150 (32), 149 (38), 95 (21), 81 (19); HRMS (EI) Found 451.3297, $C_{27}H_{41}N_5O$ requires 451.3311.

7-[(2′E,6′E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl]-tert-butoxyamino-9-methyl-7H-purinium ***12b*** *and* ***N^6-[(2′E,6′E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl]-N^6-tert-butoxy-9-methyl-9H-purin-6-amine 13b***

A mixture of *N*^{6}-*tert*-butoxy-9-methyl-9*H*-purin-6-amine **11b** (60 mg, 0.27 mmol) and allylic bromide **10** (98 mg, 0.32 mmol) in dry DMA (2.5 mL) was stirred at 50°C under N_2-atm. for 21 h and evaporated *in vacuo*. The residue was purified by flash chro-

matography on silica gel eluting with CH_2Cl_2/MeOH sat. with NH_3 (12 : 1); yield **12b** 107 mg (80%). The fractions containing isomer **13b** were combined, evaporated, and purified by flash chromatography eluting with hexane-EtOAc (1 : 1); yield **13b** 7 mg (7%).

12b

Mp 160 – 162°C, pale yellow crystals. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.95 (s, 6H, 2 × CH_3), 1.27 (s, 9H, *t*-Bu), 1.37 – 1.40 (m, 2H, CH_2), 1.49-1.53 (m, 2H, CH_2), 1.56 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.82 (s, 3H, CH_3), 1.89 (t, *J* = 5.7 Hz, 2H, CH_2), 1.95 – 2.13 (m, 8H, 4 × CH_2,), 3.84 (s, 3H, NCH_3), 5.07 – 5.10 (m, 3H, NCH_2 and CH=), 5.53 (t, *J* = 6.9 Hz, 1H, CH=), 7.83 (s, 1H, H-2), 8.99 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 16.1 (CH_3), 17.1 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.1 (CH_2), 27.6 (3 × CH_3 in *t*-Bu), 27.9 (CH_2), 28.6 (2 × CH_3), 31.4 (NCH_3), 32.7 (CH_2), 34.9 (C-6 in cyclohexene), 39.5 (CH_2), 39.8 (CH_2), 40.3 (CH_2), 47.8 (NCH_2), 77.8 (C in *t*-Bu), 110.9 (C-5), 116.0 (CH=), 122.7 (CH=), 127.0 (C=), 131.8 (C-8), 136.9 (C=), 137.0 (C=), 142.2 (C-6), 143.7 (C-4), 145.5 (C=), 154.3 (C-2); HRMS (ESI) Found 494.3836, $C_{30}H_{47}N_5O$+H requires 494.3853.

13b

Colorless oil. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.94 (s, 6H, 2 × CH_3), 1.35 – 1.39 (m, 2H, CH_2), 1.36 (s, 9H, *t*-Bu), 1.49 – 1.57 (m, 2H, CH_2), 1.53 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 1.86 (t, *J* = 6.4 Hz, 2H, CH_2), 1.87 – 1.98 (m, 8H, 4 × CH_2), 3.80 (s, 3H, NCH_3), 4.22 (br s, 1H, H_a in NCH_2), 5.00 (t, *J* = 6.4 Hz, 1H, CH=), 5.51 (t, *J* = 6.3 Hz, 1H, CH=), 5.40 (br s, 1H, H_b in NCH_2), 7.77 (s, 1H, H-2), 8.49 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 75 Hz) δ 15.9 (CH_3), 16.6 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.4 (CH_2), 27.2 (3 × CH_3 in *t*-Bu), 27.9 (CH_2), 28.6 (2 ' CH_3), 29.7 (NCH_3), 32.7 (CH_2), 34.9 (C-6 in cyclohexene), 39.6 (CH_2), 39.8 (CH_2), 40.2 (CH_2), 53.7 (NCH_2), 82.3 (C in *t*-Bu), 118.9 (CH=), 120.1 (C-5), 123.4 (CH=), 126.8 (C=), 136.0 (C=), 137.2 (C=), 139.5 (C=), 140.5 (C-8), 151.6 (C-4), 152.0 (C-2), 159.5 (C-6); MS EI *m/z* (rel.%) 493 (1), [M$^+$], 437 (21), 216 (36), 166 (36), 165 (100), 150 (15), 149 (18), 135 (23), 95 (17); HRMS (EI) Found 493.3798, $C_{30}H_{47}N_5O$ requires 493.3781.

7-[(2′E,6′E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl]-6-benzyloxyamino-9-methyl-7H-purinium ***12c*** *and N^6-[(2′E,6′E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl]-N^6-benzyloxy-9-methyl-9H-purin-6-amine* ***13c***

A mixture of *N*^{6}-benzyloxy-9-methyl-9*H*-purin-6-amine **11c** (69 mg, 0.27 mmol) and allylic bromide **10** (98 mg, 0.32 mmol) in dry DMA (2.5 mL) was stirred at 50°C under N_2-atm. for 21 h and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH sat. with NH_3 (12 : 1); yield **12c** 100 mg (70%). The fractions containing isomer **13c** were combined, evaporated, and purified by flash chromatography eluting with hexane-EtOAc (1 : 1); yield **13c** 26 mg (18%).

12c

Mp 158 – 160°C, pale yellow crystals. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 6H, 2 × CH_3), 1.37 – 1.40 (m, 2H, CH_2), 1.50 – 1.56 (m, 2H, CH_2), 1.57 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 1.88 (t, *J* = 6.1 Hz, 2H, CH_2), 1.92 – 2.11 (m, 8H, 4 × CH_2), 3.68 (s, 3H, NCH_3), 4.96 (d, *J* = 7.4 Hz, 2H, NCH_2), 5.00 (s, 2H, OCH_2), 5.07 (t, *J* = 6.4 Hz, 1H, CH=), 5.37 (t, *J* = 6.9 Hz, 1H, CH=), 7.15 – 7.21 (m, 3H, Ph), 7.31 – 7.37 (m, 2H, Ph), 7.80 (s, 1H, H-2), 8.28 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 16.1 (CH_3), 16.8 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.3 (CH_2), 27.9 (CH_2), 28.6 (2 × CH_3), 31.0 (NCH_3), 32.7 (CH_2), 35.0 (C-6 in cyclohexene), 39.5 (CH_2), 39.8 (CH_2), 40.4 (CH_2), 47.6 (NCH_2), 75.7 (OCH_2), 109.8 (C-5), 116.0 (CH=), 122.7 (CH=), 127.1 (C=), 127.1 (CH in Ph), 127.9 (2 × CH in Ph), 128.4 (2 × CH in Ph), 129.9 (C-8), 136.9 (C=), 137.0 (C=), 139.3 (C in Ph), 144.7 (C-4), 145.6 (C=), 146.4 (C-6), 156.1 (C-2); HRMS (ESI) Found 528.3687, $C_{33}H_{45}N_5O$+H requires 528.3696.

13c

Colorless oil. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 6H, 2 × CH_3), 1.36 – 1.40 (m, 2H, CH_2), 1.49 – 1.58 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.70 (s, 3H, CH_3), 1.87 (t, *J* = 6.2 Hz, 2H, CH_2), 1.95 – 2.06 (m, 8H, 4 × CH_2), 3.82 (s, 3H, NCH_3), 4.65 (d, *J* = 6.9 Hz, 2H, NCH_2), 5.07 (t, *J* = 6.3 Hz, 1H, CH=), 5.14 (s, 2H, OCH_2), 5.41 (t, *J* = 6.8 Hz, 1H, CH=), 7.15 – 7.37 (m, 3H, Ph), 7.54 – 7.57 (m, 2H, Ph), 7.80 (s, 1H, H-2), 8.49 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 16.0 (CH_3), 16.6 (CH_3), 19.5 (C-4 in cyclohexene), 19.8 (CH_3), 26.4 (CH_2), 27.9 (CH_2), 28.6 (2 × CH_3), 29.7 (NCH_3), 32.7 (CH_2), 34.9 (C-6 in cyclohexene), 39.7 (CH_2), 39.8 (CH_2), 40.2 (CH_2), 49.4 (CH_2), 77.3 (OCH_2), 118.4 (CH=), 119.4 (C-5), 123.3 (CH=), 126.8 (C=), 128.3 (2 × CH in Ph), 128.4 (CH in Ph), 129.7 (2 × CH in Ph), 136.0 (C in Ph), 136.2 (C=), 137.1 (C=), 140.3 (C=), 140.9 (C-8), 151.7 (C-4), 152.2 (C-2), 165.5 (C-6); MS EI *m/z* (rel.%) 527 (4) [M$^+$], 216 (100), 150 (32), 149 (53), 107 (40), 105 (33), 95 (34); HRMS (EI) Found 527.3612, $C_{33}H_{45}N_5O$ requires 527.3624.

(2′E,6′E)-6-Amino-9-methyl-7-[3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dien-1-yl]-7H-purinium chloride ***14***

A mixture of betaine **12b** (165 mg, 0.33 mmol), Zn (272 mg, 4.16 mmol), and AcOH (0.33 mL) in MeOH (17 mL) and water (1.7 mL) was stirred vigorously at 75°C for 19 h. The mixture was filtered and the solid washed with MeOH (17 mL). Brine (8.5 mL) and water (8.5 mL) were added and the mixture was stirred for 1 h at ambient temperature and evaporated *in vacuo*. The residue was mixed with brine (35 mL) and $CHCl_3$ (40 mL). The phases were separated and the aqueous phase was extracted with $CHCl_3$ (3 × 40 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 CH_2Cl_2/MeOH (6:1); yield 106 mg (70%), mp 181 – 183°C, colorless crystals. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.94 (s, 6H, 2 × CH_3), 1.35 – 1.39 (m, 2H, CH_2), 1.44 – 1.53 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.83 (s, 3H, CH_3), 1.87 (t, *J* = 5.9 Hz, 2H, CH_2), 1.93 – 2.06 (m, 8H, 4 × CH_2), 4.04 (s, 3H, NCH_3), 5.01 (br s, 1H, CH=), 5.44 (t, *J* = 6.8 Hz, 1H, CH=), 5.69 (d, *J* = 6.8 Hz, 2H, NCH_2), 6.98 (br s, 2H, NH_2), 8.44 (s, 1H, H-2), 10.73 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 16.1 (CH_3), 17.4 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.1 (CH_2), 27.8 (CH_2), 28.6 (2 × CH_3), 31.9 (NCH_3), 32.7 (CH_2), 34.9 (C-6 in cyclohexene), 39.5 (CH_2), 39.8 (CH_2), 40.3 (CH_2), 48.6 (NCH_2), 109.9 (C-5), 115.9 (CH=), 122.4 (CH=), 127.0 (C=), 136.9 (C=), 137.0 (C=), 145.8 (C-8), 146.7 (C=), 149.5 (C-4), 152.4 (C-6), 156.1 (C-2); HRMS (EI) Found 422.3260, $C_{26}H_{40}N_5^+$ requires 422.3278.

7-[(2′E,6′E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dienyl]-3-methyl-3H-purin-6(7H)-imine ***16***

A solution of the allylic bromide **10** (278 mg, 0.79 mmol) in dry DMA (2 mL) was added to a stirring solution of 3-methyladenine

15 (98 mg, 0.66 mmol) in dry DMA (5 mL) at 50°C under N_2-atm. After 16 h, the reaction mixture was concentrated *in vacuo*. A suspension of the residue in H_2O (1.5 mL) was made strongly basic with 10% aq. NaOH and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with brine (5 mL), dried over anhydrous K_2CO_3, and concentrated *in vacuo.* The product was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH sat. with NH_3 (8 : 1); yield 142 mg (51%), mp 98-100°C, colorless crystals. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 6H, 2 × CH_3), 1.37–1.40 (m, 2H, CH_2), 1.50–1.55 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.88 (t, *J* = 6.2 Hz, 2H, CH_2), 1.95–2.05 (m, 4H, 2 × CH_2), 2.06–2.17 (m, 4H, 2 × CH_2), 3.65 (s, 3H, NCH_3), 5.04–5.11 (m, 1H, CH=), 5.16 (d, *J* = 7.2 Hz, 2H, NCH_2), 5.47 (t, *J* = 7.1 Hz, 1H, CH=), 7.54 (s, 1H, H-8), 7.55 (s, 1H, H-2); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 16.1 (CH_3), 16.7 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.2 (CH_2), 27.8 (CH_2), 28.6 (2 × CH_3), 32.7 (CH_2), 34.3 (NCH_3), 34.9 (C-6 in cyclohexene), 39.5 (CH_2), 39.8 (CH_2), 40.2 (CH_2), 44.9 (NCH_2), 112.9 (C-5), 117.8 (CH=), 122.8 (CH=), 126.9 (C=), 136.8 (C=), 137.1 (C=), 138.9 (C-2), 143.1 (C-4), 144.7 (C-8), 144.8 (C=), 156.1 (C-6); HRMS (ESI): Found 422.3280, $C_{26}H_{39}N_5$+H requires 422.3278; Anal. Calcd.: Found: C, 73.80; H, 9.39; N, 16.53. Calc. for $C_{26}H_{36}N_5$: C, 74.07; H, 9.32; N, 16.61%.

N-{7-[(2′E,6′E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dienyl]-3-methyl-3H-purin-6(7H)-ylidene}methanamine ***17***

A mixture of imine **16** (126 mg 0.30 mmol) and MeI (0.19 mL, 3.0 mmol) in dry DMA (2 mL) was stirred at ambient temperature under N_2-atm. for 5 h, and concentrated *in vacuo*. Water (2 mL) was added and the resulting mixture was brought to pH 11 with 10% NaOH (2 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with brine (5 mL), dried over anhydrous K_2CO_3 and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH sat. with NH_3 (6 : 1); yield 47 mg (36%), mp 76–77°C, yellow crystals. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 6H, 2 × CH_3), 1.36–1.40 (m, 2H, CH_2), 1.49-1.53 (m, 2H, CH_2), 1.56 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.76 (s, 3H, CH_3), 1.87 (t, *J* = 6.2 Hz, 2H, CH_2), 1.95–2.02 (m, 4H, 2 × CH_2), 2.05–2.14 (m, 4H, 2 × CH_2), 3.21 (s, 3H, N^6CH_3), 3.62 [s, 3H, N(3)CH_3], 5.05–5.08 (m, 1H, CH=), 5.15 (d, *J* = 7.1 Hz, 2H, NCH_2), 5.42 (t, *J* = 6.8 Hz, 1H, CH=), 7.46 (s, 1H, H-2), 7.60 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 16.0 (CH_3), 16.6 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.2 (CH_2), 27.8 (CH_2), 28.6 (2 × CH_3), 32.7 (CH_2), 34.1 [N(3)CH_3], 34.3 (N^6CH_3), 34.9 (C-6 in cyclohexene), 39.5 (CH_2), 39.8 (CH_2), 40.2 (CH_2), 44.9 (NCH_2), 113.8 (C-5), 118.2 (CH=), 122.9 (CH=), 126.9 (C=), 136.7 (C=), 137.1 (C=), 135.6 (C-2), 142.7 (C-4), 145.1 (C-8), 145.2 (C=), 150.6 (C-6); HRMS (ESI): Found 436.3423, $C_{27}H_{41}N_5$+H requires 436.3434.

7-[(2′E,6′E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dienyl]-3-methyl-2,3-dihydro-1H-purin-6(7H)-imine ***18***

To a stirring solution of imine **16** (89 mg, 0.21 mmol) in 70% aqueous MeOH (3.5 mL) was added $NaBH_4$ (32 mg, 0.83 mmol). After stirring at ambient temperature under N_2-atm. for 2.5 h, the reaction mixture was concentrated *in vacuo*. The residue was partitioned between sat. aq. K_2CO_3 (10 mL) and CH_2Cl_2 (17 mL), and the aqueous phase was extracted with CH_2Cl_2 (2 × 9 mL). The organic phases were combined and dried over anhydrous K_2CO_3 and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH sat. with NH_3 (7 : 1); yield 35 mg (39%), yellow wax. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.96 (s, 6H, 2 × CH_3), 1.25–1.39 (m, 2H, CH_2), 1.42–1.53 (m, 2H, CH_2), 1.56 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.76 (s, 3H, CH_3), 1.87 (t, *J* = 6.2 Hz, 2H, CH_2), 1.95-2.02 (m, 4H, 2 × CH_2), 2.05–2.09 (m, 4H, 2 × CH_2), 2.86 (s, 3H, NCH_3), 4.29 (s, 2H, H-2), 4.72 (d, *J* = 6.5 Hz, 2H, NCH_2), 5.06-5.13 (m, 1H, CH=), 5.33 (t, *J* = 5.9 Hz, 1H, CH=), 7.21 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 16.0 (CH_3), 16.7 (CH_3), 19.5 (CH_2), 19.8 (CH_3), 26.2 (CH_2), 27.9 (CH_2), 28.6 (2 × CH_3), 32.7 (CH_2), 34.2 (NCH_3), 34.9 (C-6 in cyclohexene), 39.4 (CH_2), 39.8 (CH_2), 40.2 (CH_2), 45.1 (NCH_2), 65.1 (C-2), 104.6 (C-5), 118.6 (CH=), 122.6 (CH=), 127.0 (C=), 137.0 (2 × C=), 138.9 (C-8), 142.7 (C=), 152.7 (C-6), 157.6 (C-4); MS EI *m/z* (rel.%) 423 (4) [M^+], 216 (95), 150 (52), 149 (48), 137 (100), 122 (79), 121 (57), 95 (81); HRMS (EI) Found 423.3352, $C_{26}H_{41}N_5$ requires 423.3362.

7-[(2′E,6′E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,6-dienyl]-1,3-dimethyl-2,3-dihydro-1H-purin-6(7H)-imine ***19***

A mixture of imine **18** (104 mg 0.25 mmol) and MeI (0.06 mL, 1.0 mmol) in dry DMA (1 mL) was stirred at ambient temperature under N_2-atm. for 2.5 h. The reaction mixture was concentrated *in vacuo*, and H_2O (1.3 mL) was added. The resulting mixture was brought to pH 11 with 10% NaOH (1.3 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The CH_2Cl_2 extracts were washed with brine (4 mL), dried over anhydrous K_2CO_3 and concentrated. The residue was purified by flash chromatography eluting with CH_2Cl_2/MeOH sat. with NH_3 (6 : 1); yield 43 mg (42%), yellow oil. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.97 (s, 6H, 2 × CH_3), 1.37–1.41 (m, 2H, CH_2), 1.50–1.55 (m, 2H, CH_2), 1.55 (s, 3H, CH_3), 1.61 (s, 3H, CH_3), 1.75 (s, 3H, CH_3), 1.88 (t, *J* = 6.3 Hz, 2H, CH_2), 1.95-2.01 (m, 4H, 2 × CH_2), 2.04–2.09 (m, 4H, 2 × CH_2), 2.86 [s, 3H, N(3)CH_3], 2.95 [s, 3H, N(1)CH_3], 4.11 (s, 2H, H-2), 4.88 (d, *J* = 6.8 Hz, 2H, NCH_2,), 5.06-5.11 (m, 1H, CH=), 5.40 (t, *J* = 6.6 Hz, 1H, CH=), 7.19 (s, 1H, H-8); ^{13}C-NMR ($CDCl_3$, 300 MHz) δ 16.0 (CH_3), 16.6 (CH_3), 19.6 (CH_2), 19.8 (CH_3), 26.4 (CH_2), 27.9 (CH_2), 28.7 (2 × CH_3), 32.8 (CH_2), 33.6 [N(1)CH_3], 35.0 (C-6 in cyclohexene), 35.3 [N(3)CH_3], 39.5 (CH_2), 40.0 (CH_2), 40.3 (CH_2), 44.9 (CH_2), 71.2 (C-2), 107.2 (C-5), 118.6 (CH=), 123.0 (CH=), 126.9 (C=), 137.0 (C=), 137.3 (C=), 137.8 (C-8), 141.9 (C=), 154.7 (C-4), 155.3 (C-6); MS EI *m/z* (rel.%) 437 (7) [M^+], 232 (100), 137 (65), 122 (54), 121 (26), 95 (40); HRMS (EI) Found 437.3504, $C_{27}H_{43}N_5$ requires 437.3518.

Growing of cultures

E. coli was grown on Tryptone-soya agar (TSA; Oxoid, Basingstoke, Hampshire, UK). Non-nutrient agar (NNA) used for growing protozoal cultures prior to testing was made as follows: Bacteriological agar (Oxoid, 15 g) was dissolved by autoclaving in 1 L Page's Amoeba Saline (PAS). PAS was made by mixing 5 mL of solutions 1 (g/L: NaCl, 24; $MgSO_4 \cdot 7\,H_2O$, 0.8; $CaCl_2 \cdot 6\,H_2O$, 1.2) and 2 (g/L: Na_2HPO_4, 28.4; KH_2PO_4, 27.2) and adding dH_2O to 1000 mL. *E. coli* was grown by plating into TSA followed by incubation at 35 ± 2°C for 18–24 h. Cells were harvested from plates using a TECRA1 ENVIROSWAB (Tecra International Pty Ltd, French Forest, New South Wales) and a thick suspension (20 mL) was made in physiological saline (FS; 0.9% NaCl). The suspension was pasteurized prior to use by heating to 60°C for 25–30 min. The whole surface of an NNA plate was moistened with the pasteurized *E. coli* suspension using an ENVIROSWAB. One drop of the original protozoal culture or a loopful of growth from an NNA/*E. coli* culture was placed in the centre of the plate. The plate was

then sealed using clear tape and incubated for 36 – 48 h at 30 ± 2°C. After incubation, 1 – 2 mL of FS was added to the plate and the growth was suspended using a plate spreader. The cell suspension was spun down in a microcentrifuge for 5 min. at 1000 × g and the supernatant containing *E. coli* was removed. The protozoal pellet was then suspended in 2 mL PS and spun for a further 3 min. at 750 × g. After removal of the supernatant the pellet was dissolved in 1 ml PS and spun again at 500 × g for 3 min. Finally the supernatant was removed and the protozoa were suspended in 0.5 – 1.0 mL FS containing heat-killed *E. coli* (OD_{530} = 0.12) grown as described above. Additional bacterial suspension was added as required to achieve a trophozoite density of about 1.0×10^5 trophozoites/mL as adjudged by counting using a microscope. The viability of the cells was examined by addition of TB at 0.04% prior to counting.

Preparation of antimicrobials

Agelasine analogs **12** and **14** and agelasimine analogs **16** – **18** were prepared as stock solutions of 5120 μg/mL according to the recommendations of the National Committee for Clinical Laboratory Standards [36]. Sterile deionized water (benzalkonium chloride) or dimethylsulfoxide (agelasines) was used as the solvent, and sterile deionized water was used as the dilutant. Serial two-fold dilutions of the test substances were made in the range 4 – 128 μg/L. This gave after addition of the inoculum a test range of 2 – 64 μg/L. Stock solutions of agelasines were stored in polyethylene vials at −80°C until the day of use. Benzalkonium chloride was freshly prepared for each experiment.

Antimicrobial testing

Susceptibility testing was performed using a microdilution technique. Dilutions of the antimicrobial (50 μL) were added in duplicate to a microtiter plate. To each well was added 50 μL of the inoculum. 100 μL inoculum without antimicrobial was used as a positive growth control. After inoculation, plates were sealed with tape, packaged in plastic bags, and incubated aerobically at 30 ± 20C for 36 – 48 h. After incubation, the contents of each well was thoroughly mixed using an automatic pipette, taking care to scrape the walls of the wells with the tips to dislodge cells. TB was added to samples at 0.04% and the cells were examined in the microscope in several 10 μL portions (approximately 500 cells based on the inoculum concentration). The minimum trophocidal concentration (MTC) was read as the lowest concentration of antimicrobial agent producing all blue trophozoites. In practice, in deciding the MTC a limit of two white trophozoites was set per drop. Tests were repeated at least once with fresh samples and a fresh inoculum.

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