Mallotojaponins B and C: Total Synthesis, Antiparasitic Evaluation and Preliminary SAR Studies

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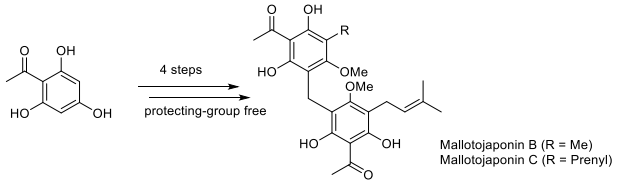
Supporting Information Placeholder



ABSTRACT: The first total syntheses of mallotojaponin B and C as well as several analogues have been achieved. Biological evaluation of the synthetized compounds against *P. falciparum* and *T. Brucei* have also been carried out.

Malaria is a tropical and subtropical disease caused by parasites of the *Plasmodium* genus carried by *Anopheles* mosquitoes, among which the most lethal form is caused by *Plasmodium falciparum*. Global estimations show that between 300 and 600 million people are infected and that about a million die every year from this infection. The discovery of new drugs for fighting this epidemic is essential, since resistances have emerged to most of the currently employed treatments. Natural products have constituted a seemingly endless source of inspiration for the development of drugs, as showcased by artemisinin, which is now the World Health Organization's recommended first-line treatment against malaria and for which discovery the Nobel Prize of Medicine was awarded in 2015. However, resistance to artemisinin has recently been reported and is spreading,[1] thus increasing the urgent need for developing alternative treatments. Mallotojaponins B **1** and C **2** are phloroglucinol dimers that have recently been isolated from a Madagascar euphorbiacea, *Mallotus oppositifolius*, that display potent antiplasmodial activities (Figure 1).[2]

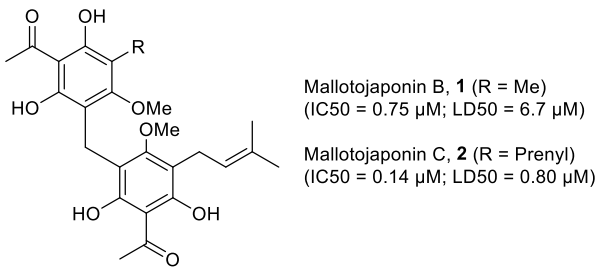
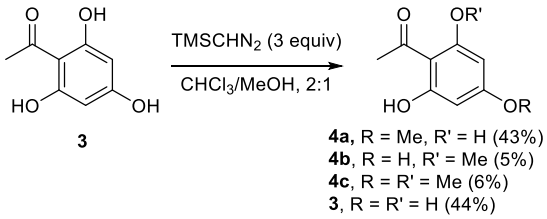


Figure 1 Mallotojaponins B and C: structure and activities against *P. falciparum* Dd2 (chloroquine/mefloquine resistant strain)

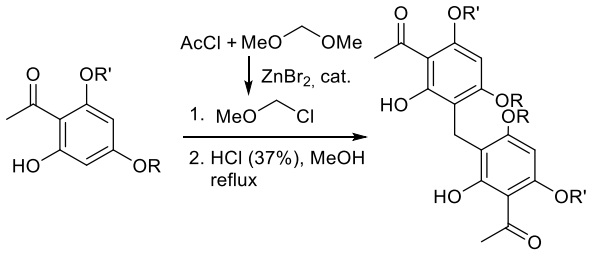
In particular, mallotojaponin C displays both cytocidal and gametocidal activities (IC50 = 0.14 µM and 3.6 µM, respectively) on *Plasmodium falciparum*, making it a good candidate for further investigation. Following an ongoing interest in the development of novel antiparasitic compounds,[3] a program was initiated, aimed at the synthesis of these two compounds and at the further evaluation of their biological activities. In particular, based on the difference of activity between mallotojaponins B and C assessing the influence of the number of prenyl units was considered. Since the target compounds are dimeric, two strategies could be envisioned: functionalization followed by dimerization, or vice versa. Based on our previous experience with the dimerization of phloroglucinol derivatives,[4] the latter route was initially deemed more convergent, especially towards homo-dimeric adducts. Thus, phloroacetophenone **3** was first treated with trimethylsilyldiazomethane[5] leading predominantly to the desired para-*O*-methylated product **4a** (77% yield based on recovered starting material – brsm), along with minor amounts of ortho-*O*- and *O,O'*-dimethylated compounds **4b** and **4c**, respectively (Scheme 1).

Scheme 1 Methylation of phloroacetophenone 3



The first set of mallotojaponin analogues was obtained by subjecting all three acetophenones **4a-c** to methylenation conditions using MOMCl (generated *in situ* from dimethoxymethane) followed by heating with hydrochloric acid (Scheme 2).[4] The corresponding bridged dimers **5a-c** were all obtained with excellent yields; however further elaboration of these substrates to mallotojaponins, especially *C*-prenylation, proved extremely laborious.[6]

Scheme 2 Methylenation of acetophenones 4a-c

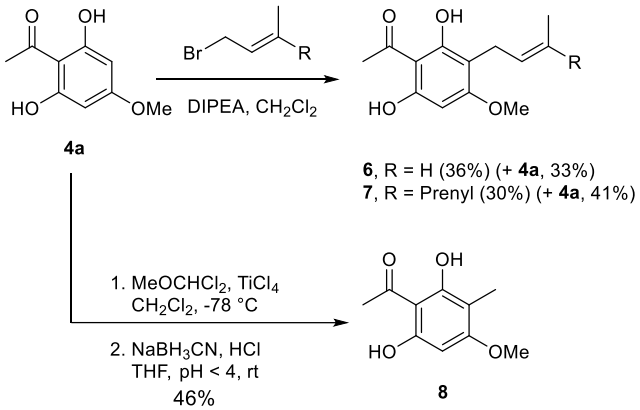


4a, R = Me, R' = H
4b, R = H, R' = Me
4c, R = R' = Me

5a, R = Me, R' = H (90%)
5b, R = H, R' = Me (86%)
5c, R = R' = Me (82%)

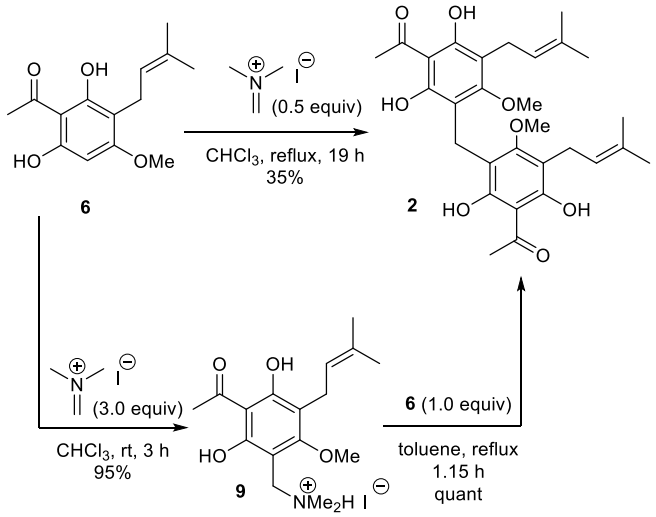
At this stage, the strategy was reconsidered, and the functionalized monomers were targeted first before carrying out the dimerization, keeping in mind that the ideal protocol should grant access to both homo- and hetero-dimeric compounds. Although well documented,[7] selective *C*-prenylation of compound **4a** was found to be the major hurdle to overcome in this endeavor. Many multistep strategies such as selective TBS-mono protection/*O*-prenylation[8] or selective *O*-prenylation followed by Claisen rearrangement, Pd-catalyzed *O*-3,3-dimethylallylation/sigmatropy[9] were evaluated to little or no avail. Finally, direct *C*-prenylation with prenyl bromide,[10] in the presence of Hünig's base in dichloromethane offered the best compromise (Scheme 3).[11] Indeed a reasonable 53% (brsm) yield of prenylated phenol **6** could be attained and these conditions also enabled the isolation of the corresponding geranyl adduct **7** (51% brsm). Synthesis of methylated monomer **8** has previously been reported by a three-step sequence starting with 2,4,6-trihydroxybenzaldehyde.[12] Nevertheless, direct access from readily available **4a** was thought to be more practical. This was achieved by a two-step sequence involving formylation with dichloro(methoxy)methane[13] followed by reduction using sodium cyanoborohydride under acidic conditions.[14]

Scheme 3 Prenylation, geranylation and methylation of acetophenone 4a



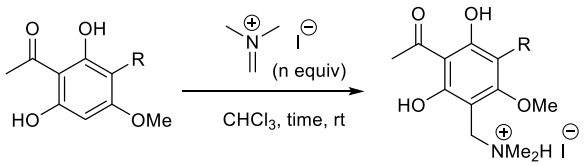
With these three functionalized monomers in hand the dimerization reaction was attempted, which was unsuccessful using the MOMCl protocol. After screening various formaldehyde equivalents Eschenmoser's salt was eventually considered, as it was briefly reported by Minassi and Appendino to be suitable promoter for this kind of transformation in the synthesis of arzanol.[15] Reacting prenylated monomer **6** with half an equivalent of the methylene salt allowed the synthesis of mallotojaponin C **2** in 35% yield (Scheme 4). In order to improve the efficiency of this final step the reagent-to-starting material ratio was increased. Interestingly, when three equivalents of reagents were used exclusive formation of the dimethylammonium salt took place, giving **9** in 95% yield. This platform could in turn be reacted with one equivalent of the starting monomer **6** to give the desired natural product in quantitative yield.[16]

Scheme 4 Methylenation of prenylated acetophenone 6



This encouraging result prompted us to synthesize the two remaining dimethylammonium salts **10** and **11** from the corresponding geranylated and methylated phenols **7** and **8**, respectively. This was achieved by reacting each monomer with three equivalents of Eschenmoser's salt in chloroform at room temperature (Scheme 5).

Scheme 5 Synthesis of dimethylammonium salts 10 and 11



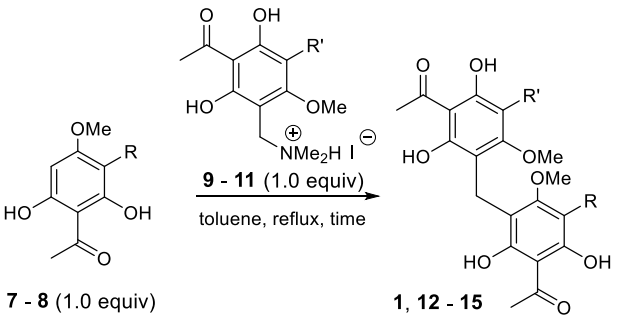
7, R = Geranyl
8, R = Methyl

10, R = Geranyl (82%)
(n = 3.0 , *t* = 1.15 h)
11, R = Methyl (quant)
(n = 1.0 , *t* = 1.15 h)

With both the phenols and the ammonium salts now available an efficient access to either homo- or heterodimers was within reach. First, digeranyl and dimethyl adducts **12** and **13** could indeed be obtained with excellent yields by reacting together the similarly substituted phenols and salts (Table 1, entries 1, 2). The latter compound **13** is actually mallotophenone, another naturally-occurring phloroglucinol dimer, that was also isolated from *Mallotus* species.[2,17] Heterodimers could in turn be obtained with good yields (Table 1, entries 3–5) by reacting together phenols and salts bearing different substituents such as

geranyl/prenyl **14**, geranyl/methyl **15** and prenyl/methyl (i.e. mallotojaponin B, **1**)

Table 1. Synthesis of dimers 12 –15 and 1[a]



entry	R	R'	time (min)	dimer, yield (%)
1	**7**, geranyl	**10**, geranyl	90	**12**, 98
2	**8**,methyl	**11**, methyl	50	**13**, 94
3	**7**, geranyl	**9**, prenyl	75	**14**, 83
4	**8**, methyl	**10**, geranyl	60	**15**, 68
5	**8**, methyl	**9**, prenyl	45	**1**, 62

[a] isolated yields

Following our initial objective, the anti-malarial activity (Table 2, column 3) of the various synthesized compounds was first evaluated on the intraerythrocytic stage of *P. falciparum*, starting with natural products **1**, **2** and **13** (Table 2, entries 1–3). While the measured IC_{50}'s are slightly higher than the ones reported[2] (on a different strain), the same order of potency prevails, with diprenylated mallotojaponin C (Table 2, entry 2) being the most active, followed by mono-prenylated mallotojaponin B (Table 2, entry 1) and non-prenylated mallotophenone (Table 2, entry 3). Unsubstituted dimers with different methoxylation patterns **5a–c** (Table 2, entries 4–6) display at best modest activity against *P. falciparum*. Only **5a**, which structurally most resembles **13**, displays an activity below 50 µM (Table 2, entry 4). Micromolar activities were restored with geranyl-substituted dimers **12**, **14** and **15** (Table 2, entries 7–9). The most active compound of this latter series is mono-geranylated **15** (IC_{50} = 1.0 µM) (Table 2, entry 9). In order to further probe the influence of the prenyl units, diisopentyl dimer **16** and diallyl dimer **17** were also synthesised and evaluated.[18] Both analogues were found to be more active (Table 2, entries 10 and 11) than mallotojaponin B **1**, yet less so than mallotojaponin C **2**, thus demonstrating the activating influence of the prenyl units. Finally, several monomers and salts were also tested for their anti-*plasmodium* activity (Table 2, entries 12-16). All monomers but methylated phenol **8** exhibited some inhibitory potency, with the most active of these (**7**, IC_{50} = 4.4 µM) bearing a geranyl unit (Table 2, entry 13). Both dimethylammonium salts **9** and **10** were found to be active (Table 2, entries 15 and 16), although this could be mostly due to their intrinsic reactive nature. The scope of this study was further extended by screening all compounds against the bloodstream forms of *T. brucei*, the parasite responsible for trypanosomiasis in humans and cattle (column 4). Interestingly, an activity profile that almost mirrors that found against *P. falciparum*, could be evidenced with the three most active compounds (Table 2, entries 2, 9 and 10) also being mallotojaponin C (**2**), methyl/geranyl dimer **15** and saturated dimer **16** (IC_{50} = 0.45 µM, 0.53 µM and 0.77 µM, respectively). Finally, considering the apparent importance of the prenyl units for activity, the substrates were also tested as potential inhibitors of *T. brucei* protein farnesyl transferase (Table 2, column 5). A moderate correlation could thus be observed as mallotojaponin C (**2**) and methyl/geranyl dimer **15** were found to be good inhibitors of the enzyme, in the micromolar range (IC_{50} = 2.0 µM and 5.2 µM, respectively). Yet, in this regard, saturated analogue **16** still appears as a relatively potent compound (IC_{50} = 4.4 µM) (Table 2, entry 10).

Table 2 Evaluation of antiparasitic and enzyme inhibitory activities [a]

entry	compound	*P. falciparum*[b]	*T. brucei*[c]	farnesyl transferase[d]
1	**1**	3.4 ± 0.2	1.0 ± 1.0	>30
2	**2**	0.75 ± 0.11	0.45 ± 0.01	2.0 ± 0.3
3	**13**	12.9 ± 4.6	7.9 ± 0.4	>30
4	**5a**	17.6 ± 4.3	10 ± 1.1	25.5 ± 2.9
5	**5b**	>50	>50	>30
6	**5c**	>50	>50	>30
7	**12**	6.0 ± 0.4	3.7 ± 0.2	>30
8	**14**	2.1 ± 1.3	1.1 ± 0.04	14.4 ± 2.4
9	**15**	1.0 ± 0.8	0.53 ± 0.03	5.2 ± 0.5
10	**16**	2.0 ± 1.3	0.77 ± 0.08	4.4 ± 0.7
11	**17**	4.1 ± 0.9	2.0 ± 0.2	22.8 ± 6.9
12	**6**	7.4 ± 1.1	27 ± 0.8	>30
13	**7**	4.4 ± 1.3	8.5 ± 0.2	>30
14	**8**	>50	>50	>30
15	**9**	3.5 ± 0.5	4.1 ± 0.1	>30
16	**10**	3.7 ± 0.1	8.4 ± 0.6	>30

[a] IC50 in µM. [b] Chloroquine-resistant *P. falciparum* FcB1/Colombia strain. [c] *T. brucei brucei* strain 93. [d] *T. brucei brucei* protein farnesyl transferase.

Overall, these synthetic efforts culminated in the first total syntheses of three natural products that were recently isolated from *Mallotus oppositifolius*: Mallotojaponins B and C as well as mallotophenone.[2] Biological evaluation of these compounds and of various analogues confirmed their promising antimalarial activity and uncovered an interesting trypanocidal activity. Preliminary SAR studies showed the importance of the two prenyl units and of the dimeric structure. Further synthetic and biological studies are currently underway to explore the full therapeutic potential of this family of compounds and will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Synthetic and biological protocols, copies of NMR spectra (PDF)

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ACKNOWLEDGMENT

We thank the CNRS for financial support and the Institut de Chimie des Substances Naturelles, for financial support and a fellowship for T. D. G.

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(6) Prenylation of **5a** did provide a minor amount of mallotojaponin C (ca 15%) that was difficult to isolate and purify.
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(16) The same strategy has very recently been applied to the synthesis of rottlerin, see: Hong, K. K. C.; Ball, G. E.; Black, D. StC.; Kumar, N. *J. Org. Chem.* **2015**, *80*, 10668.
(17) Mallotophenone was first isolated from *Mallotus japonicus*, see: (a) Arisawa, M.; Fujita, A.; Suzuki, R.; Hayashi, T.; Morita, N.; Kawano, N.; Koshimura, S. *J. Nat. Prod.* **1985**, *48*, 455; however the synthesis of this compound had been previously reported, see: (b) McGookin, A.; Robertson, A.; Simpson, T. H. *J. Chem. Soc.* **1951**, 2021.
(18) Diisopentyl compound **16** was obtained by hydrogenation on Pd/C of mallotojaponin C, and diallyl compound **17** was prepared in three steps from **4a**, see Supporting Information for details.