One-step Multigram-scale Biomimetic Synthesis of Psiguadial B

Christopher G. Newton,[a] Duc N. Tran,[a],[b] Matthew D. Wodrich[c] and Nicolai Cramer*,[a]

Abstract: A gram-scale synthesis of psiguadial B, a purported inhibitor of human hepatoma cell growth, has been achieved in one-step via a biomimetic three-component coupling of caryophyllene, benzaldehyde and diformylphloroglucinol. This cascade reaction is catalyzed by *N,N'*-dimethylethylenediamine, and proceeds at ambient temperature to generate four stereocenters, two rings, one C–O bond, and three C–C bonds. Combined computational and experimental investigations suggest the biosynthesis of the natural product is non-enzyme mediated, and is the result of a Michael addition between caryophyllene and a reactive *ortho*-quinone methide, followed by two sequential intramolecular cationic cyclization events.

Psidium guajava (the common guava) is a small, fruit-bearing tree cultivated in tropical and subtropical climates throughout the world. Constituents of the plant have long found application in traditional medicines across Central American, Caribbean, African, and Asian cultures for the treatment of various conditions, including diarrhea and hyperglycemia.[1] Several caryophyllene (**1**) derived natural products have been identified in guava leaf extracts (Figure 1),[2] including psiguadial B (**2**), a complex meroterpenoid first isolated by Ye and co-workers in 2010.[2a] Preliminary biological testing of **2** revealed significant antitumor activity, notably exhibiting selectivity for HepG2 over HepG2/ADM cells (IC_{50} values of 45.62 ± 1.41 nM and 25.09 ± 0.21 μM, respectively), suggesting it may be an inhibitor of P-glycoprotein. The central bicyclo[4.3.1]decane ring system of psiguadial B, *trans*-fused to both a cyclobutane and a highly functionalized chromane, represents a considerable synthetic challenge. Indeed, only one total synthesis of the molecule has been reported. In 2016 the Reisman group disclosed a 16 step abiotic approach,[3] obtaining the natural product on a <10 mg scale (comparable to quantities attained through isolation; 25 mg from 20 kg of dried leaves[2a]). As part of our previously disclosed studies concerning the biomimetic syntheses of several related aromadendrene sesquiterpenoids[4] we became interested in the biosynthetic origins of psiguadial B (**2**). We speculated that despite its substantial complexity it may be accessible via a cascade reaction between caryophyllene (**1**) and a suitable phloroglucinol-derived coupling partner. Herein, we report the successful realization of this strategy, achieving a one-step synthesis of psiguadial B (**2**), while also circumventing the need for preparative HPLC to secure a practical, multigram-scale purification. Furthermore, with the aid of combined experimental and computational studies we propose a mechanism for its biosynthesis.

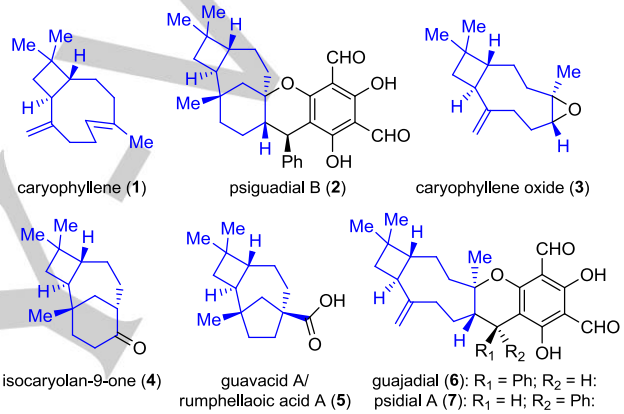

Figure 1. Representative caryophyllene-derived natural products isolated from the leaves of *Psidium guajava*.

Collado et al. demonstrated caryophyllene oxide (**3**) can be converted into a mixture of isocaryolane-9-one (**4**) and the (presumed) aldehyde biosynthetic precursor to guavacid A/rumphellaoic acid A (**5**) in a two-step sequence incorporating a one-pot, acid-promoted cationic alkene cyclization/1,2-migration.[5] More recently, Lee and coworkers synthesized guajadial (**6**) and psidial A (**7**) via a biomimetic hetero-Diels–Alder (hDA) reaction between **1** and a reactive *ortho*-quinone methide (*o*-QM).[6] Drawing inspiration from these studies, as well as reports concerning skeletal rearrangements of caryophyllene (**1**),[7] we initially considered three plausible mechanistic scenarios for the biosynthesis of psiguadial B (**2**) (Scheme 1). Our first proposal begins with an acid-catalyzed isomerization of the more reactive endocyclic alkene of caryophyllene (**1**) to generate bis-exocyclic diene **8** (Scheme 1, part I). Further acid treatment may generate carbocation **9**, which appears pre-disposed towards a diastereoselective intramolecular cationic alkene cyclization. Notably, addition of water to the resultant carbocation **10** would provide the natural product isocaryolan-8-ol, a transformation that has been successfully conducted in the laboratory by treatment of caryophyllene with acidic aluminium oxide.[8] Regioselective deprotonation of tricyclic intermediate **10** to known terpene **11**,[9] followed by trapping in a hDA reaction with *o*-QM's **12** (via either geometrical isomer[10]), could yield 9-*epi*-psiguadial B (**13**).

[a] Dr. C. G. Newton, Dr. D. N. Tran, Prof. Dr. N. Cramer
Laboratory of Asymmetric Catalysis and Synthesis
EPFL SB ISIC LCSA, BCH 4305
1015 Lausanne (Switzerland)
E-mail: nicolai.cramer@epfl.ch
Homepage: http://isic.epfl.ch/lcsa
[b] Dr. D. N. Tran
Present address: Janssen Pharmaceutical N.V.
API Small Molecule Development
Turnhoutseweg 30, B-2340 Beerse (Belgium)
[c] Dr. M. D. Wodrich
Laboratory for Computational Molecular Design
EPFL SB ISIC LCMD, BCH 5121
1015 Lausanne (Switzerland)
E-mail: matthew.wodrich@epfl.ch

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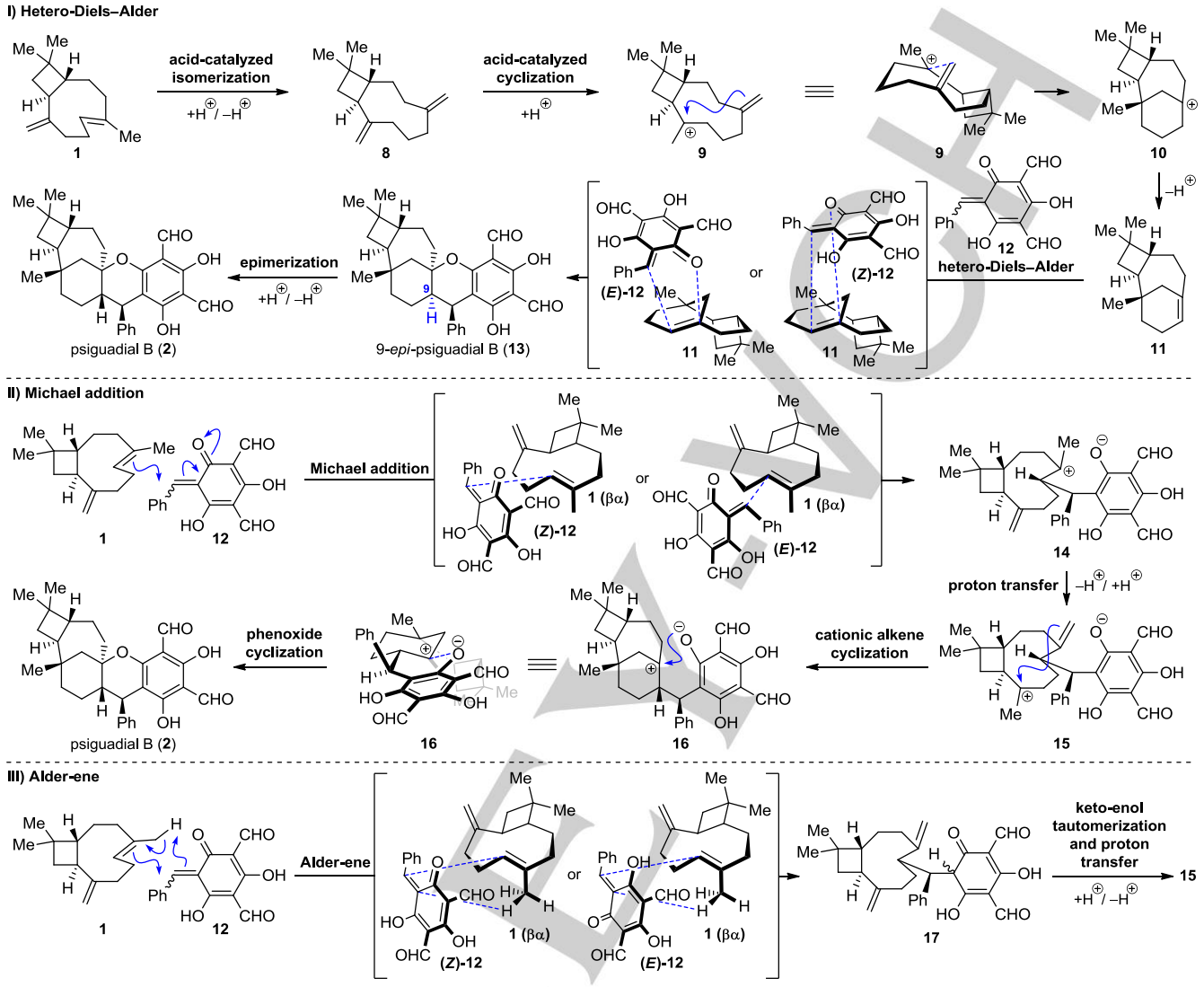
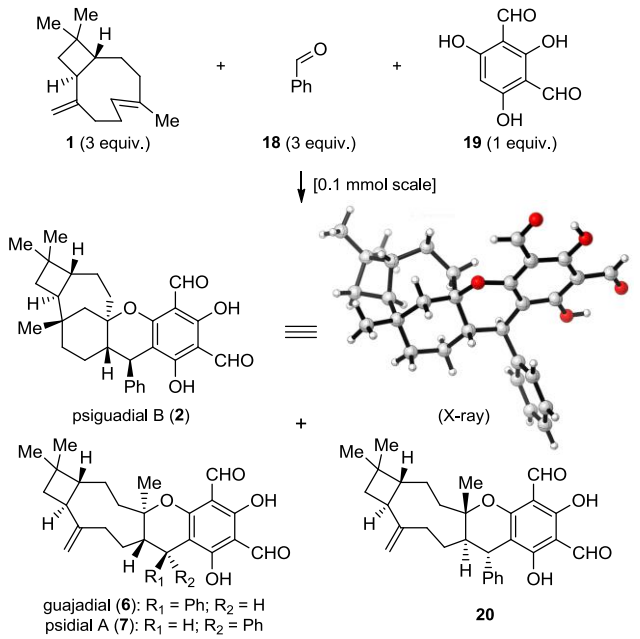


Scheme 1. Proposed biosynthetic routes to psiguadial B (**2**), incorporating either a hDA reaction (I), Michael addition (II), or Alder-ene reaction (III).

Finally, isomerization of the *cis*-fused chromane ring junction delivers the natural product. Alternatively, psiguadial B (**2**) may be the result of a Michael addition between caryophyllene (via its predominant βα-conformation[7]) and *o*-QM's **12** (Scheme 1, part II). A formal proton transfer converts **14** to **15**,[11] which could subsequently cyclize in an analogous fashion proposed for the conversion of **9** to **10**. Intramolecular phenoxide cyclization of **16** then sets the final stereocenter, and completes the synthesis.[12] Our third proposal is closely related to the Michael addition pathway, but instead involves the generation of an inconsequential mixture of diastereoisomers **17** through an Alder-ene reaction between caryophyllene and *o*-QM's **12** (Scheme 1, part III). Tautomerization, followed by a proton transfer, intercepts intermediate **15**.

Synthetic studies towards psiguadial B (**2**) were initiated employing ethylenediammonium diacetate (EDDA) as a catalyst for *in situ* formation of *o*-QM's **12** via Knoevenagel condensation[13] of benzaldehyde (**18**) and diformylphloroglucinol (**19**)[14] (Table 1). Conducting the reaction in either *i*PrOH or trifluoroethanol (TFE) at 60 °C provided a mixture of known hDA adducts, guajadial (**6**), psidial A (**7**), and **20**.[6a] However psiguadial B (**2**) could not be detected (entries 1 and 2). We reasoned that a more polar and less nucleophilic solvent may promote the formation of the proposed cationic intermediates. Indeed, switching to hexafluoroisopropanol (HFIP) yielded psiguadial B (**2**) in a 4.6% ^{1}H NMR yield (entry 3). A reduction in the reaction temperature resulted in a modest improvement in yield (entry 4), whereas a shift to nonafluoro-*tert*-butyl alcohol (F_9-*t*BuOH) as solvent proved mildly detrimental (entry 5). Altering the concentration of the reaction had little effect (entries 6 and 7). However, the consequences of changing the EDDA loading were more pronounced (entries 8 and 9), likely highlighting the importance of the relative *o*-QM concentration

Table 1. Optimization of the biomimetic synthesis of psiguadial B (**2**).



#	Catalyst (20 mol %)	Solvent (0.5 M)	Time (h)	Temp. (°C)	Combined Yield (%)[a]	Yield of **2** (%)[b]
1	EDDA	*i*PrOH	1	60	21	0
2	EDDA	TFE	1	60	20	0
3	EDDA	HFIP	1	60	19	4.6
4	EDDA	HFIP	6	r.t.	29	5.8
5	EDDA	F_9-*t*BuOH	6	r.t.	20	4.2
6	EDDA	HFIP (0.1 M)	6	r.t.	19	4.1
7	EDDA	HFIP (1.0 M)	6	r.t.	21	5.2
8	EDDA[c]	HFIP	6	r.t.	0	0
9	EDDA[d]	HFIP	6	r.t.	15	3.5
10	ethylenediamine	HFIP	4	r.t.	19	3.4
11	NaOAc[e]	HFIP	48	r.t.	10	0
12	*n*Bu-NH_2[e]	HFIP	48	r.t.	<2	0
13	piperidine[e]	HFIP	48	r.t.	<2	0
14	piperazine	HFIP	18	r.t.	34	9.3
15	TMEDA	HFIP	48	r.t.	34	7.5
16	DMEDA	HFIP	18	r.t.	28	10.3
17	-	HFIP	18	r.t.	0	0
18	DMEDA	HFIP	18	r.t.	37[g]	10.4 (8.0[h])

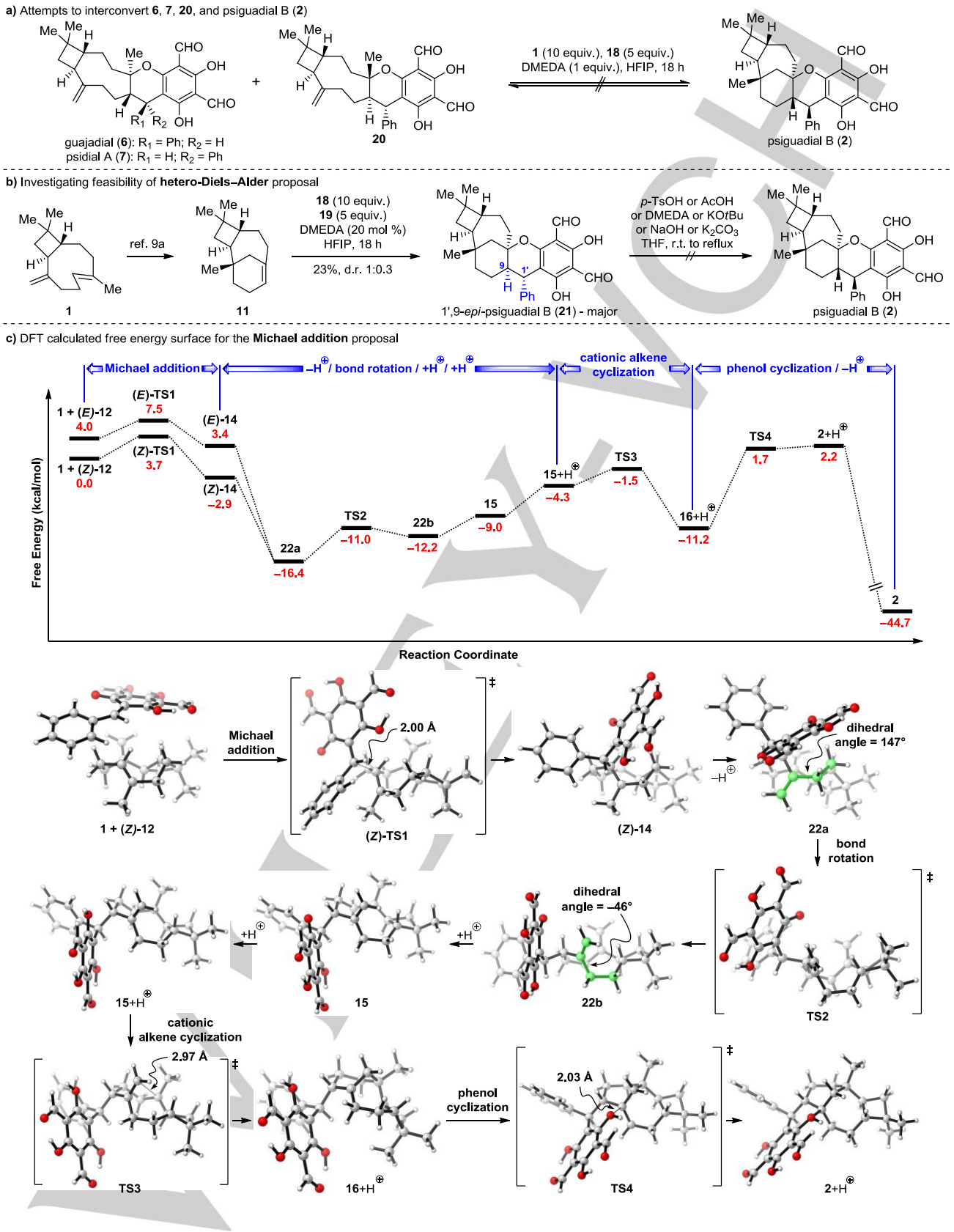
[a] Combined yields of **2**, **6**, **7** and **20**, determined by ^{1}H NMR with an internal standard. [b] Determined by ^{1}H NMR with an internal standard. [c] 100 mol %. [d] 5 mol %. [e] 40 mol %. [f] 55 mmol scale, 6.0 equiv. of caryophyllene, **19** added in 4 batches, each 0.25 equiv. and 1.5 h apart. [g] Ratio of **2**:**6**:**7**:**20** = 28:37:25:10, determined by ^{1}H NMR. [h] Isolated yield, 2.08 g. EDDA – ethylenediammonium diacetate; TFE – trifluoroethanol; HFIP – hexafluoroisopropanol; TMEDA – tetramethylethylenediamine; DMEDA – *N,N'*-dimethylethylenediamine

(e.g. competing polymerization). Evaluation of alternative catalyst systems was undertaken next. The two components of EDDA, ethylenediamine and NaOAc, were screened independently (entries 10 and 11). The former provided the target in a reduced 3.4% ^{1}H NMR yield, whereas the latter only furnished hDA adducts **6**, **7**, and **20**. Monoamines, such as *n*Bu-NH_2 and piperidine, were ineffective catalysts (entries 12 and 13), prompting us to screen additional diamine derivatives. Although piperazine and tetramethylethylenediamine (TMEDA) were both competent (entries 14 and 15), *N,N'*-dimethylethylenediamine (DMEDA) proved superior, providing psiguadial B (**2**) in a 10.3% ^{1}H NMR yield (entry 16). Finally, a control reaction conducted in the absence DMEDA, under otherwise identical conditions, failed to generate any identifiable adducts (entry 17).

Although moderate yielding, the brevity and operational simplicity of our synthetic route (reaction run open to air and at ambient temperature, commercial reagents used without further purification), encouraged us to explore a preparative synthesis of the natural product. The DMEDA catalyzed conditions scaled well with an increased caryophyllene (**1**) loading and the batch-wise addition of **19**, ultimately generating psiguadial B (**2**) in a 10.4% ^{1}H NMR yield on 55 mmol scale (entry 18). Purification was facilitated by treatment of the crude reaction mixture with ozone to selectively decompose **6**, **7** and **20** [see supporting information (SI) for details], allowing the isolation of psiguadial B (**2**) on 2.08 g scale (8.0% yield) without the need for HPLC purification.[15] Furthermore, we successfully obtained the first X-ray crystal structure of **2** to unequivocally confirm its identity.

Insight into the mechanism of this cascade transformation was acquired through additional synthetic studies (Scheme 2a and 2b). First we confirmed that hDA adducts **6**, **7**, **20**, and psiguadial B (**2**) do not interconvert under the reaction conditions (Scheme 2a). Next, we shifted our attention toward exploring the viability of our hDA proposal outlined in Scheme 1, part I. Potential dienophile **11** was accessed in two steps from caryophyllene (Scheme 2b).[9a] Reaction of **11** with *o*-QM's **12** under unoptimized conditions provided a mixture of 1',9-*epi*-psiguadial B (**21**), and an unassigned minor diastereoisomer, in a combined 23% yield. However, no trace of psiguadial B (**2**) was observed. Furthermore, neither acid nor base treatment of this mixture afforded **2**, indicating the hDA pathway is unlikely responsible for the formation of psiguadial B.

Efforts directed towards experimentally verifying the Michael addition (Scheme 1, part II) or Alder-ene (Scheme 1, part III) proposals proved unsuccessful. We therefore explored the feasibility of these mechanisms using DFT computations, conducted at the PBE0[16]-dDsC[17]/TZ2P//PBE0-D3(BJ)[18]/def2-SVP theoretical level (Scheme 2c). All attempts to computationally observe a concerted Alder-ene reaction between caryophyllene (**1**) and *o*-QM's **12** were unsuccessful. In comparison, the Michael addition of caryophyllene (**1**) is computed to be exergonic with either *o*-QM geometric isomer, **(*Z*)-12** or **(*E*)-12**, leading to conformational isomers **(*E*)-14** and **(*Z*)-14** (see the SI for the higher energy **(*E*)-12** pathway structures). Moreover, similar free energy barriers are observed for both transition states [**(*E*)-TS1** = 3.5, and **(*Z*)-TS1** = 3.7 kcal/mol], indicating both isomers likely participate. Direct conversion of Michael addition adducts **(*E/Z*)-14** to putative alkene cyclization precursor **15** through an intramolecular proton transfer is not geometrically possible. Computations indicate the requisite bond rotation energy of the tertiary methyl substituent would be in excess of 60 kcal/mol, and therefore unlikely to



Scheme 2. Combined computational and synthetic studies into the mechanism of psiguadial (**2**) formation.

occur. Thus, a three-step deprotonation/bond rotation/protonation sequence [i.e. **(*E*/*Z*)-14** → **22a** → **22b** → **15**] is preferred. Subsequent cationic alkene cyclization of **15** is not favored as a result of competing proton transfer between the tertiary methyl substituent and phenoxide. Rather, protonation of the phenoxide to generate **15**+H^+, followed by cyclization through **TS3**, generates the bicyclo[4.3.1]decane ring system of **16**+H^+. The second cyclization event, in this case to close the chromane ring, is computed to be both rate determining (ΔG = 12.9 kcal/mol) and endergonic. Finally, deprotonation of **2**+H^+ delivers psiguadial B. Overall the Michael addition pathway is calculated to be exergonic by almost 45 kcal/mol. This study supports its plausibility as a biosynthetic proposal, and given the inherent diastereoselectivity of the cascade transformation we speculate the process is non-enzyme mediated in nature.

In summary, we have synthesized psiguadial B (**2**) in one-step via a DMEDA catalyzed biomimetic three-component coupling of caryophyllene (**1**), benzaldehyde (**18**) and diformylphloroglucinol (**19**). This cascade reaction involves the construction of two rings, four stereocenters and can be conducted on multigram-scale, effectively demonstrating the advantage biomimetic strategies towards natural products can offer.[19] Combined experimental and computational studies prompt us to propose the biosynthesis of **2** is non-enzyme mediated, and proceeds via a Michael addition between caryophyllene and an *o*-QM, followed by a series of proton transfers and cationic cyclizations. The ability to readily access gram quantities of psiguadial B (**2**) will no doubt facilitate further biological appraisal of the natural product.

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Keywords: biomimetic synthesis • natural products • meroterpenoids • *ortho*-quinone methide • caryophyllene

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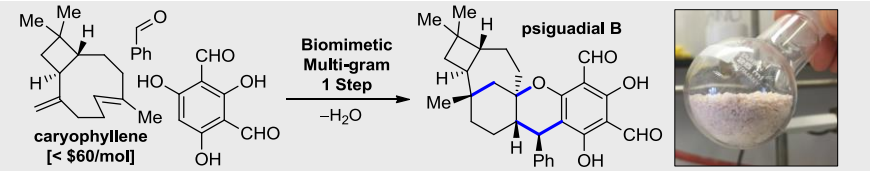

*C. G. Newton, D. N. Tran, M. D. Wodrich, N. Cramer**

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A one-step multigram-scale synthesis of psiguadial B has been achieved using a biomimetic three-component coupling, generating three C–C bonds, one C–O bond, two rings, and four stereocenters. Combined synthetic and computational experiments suggest the reaction proceeds via a Michael addition of caryophyllene to an *in situ* generated *ortho*-quinone methide, followed by two sequential cationic cyclization events.