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Total Synthesis of RamonaninsA–D

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Total Synthesis of Ramonanins A–D**

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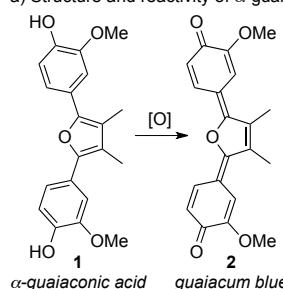
Abstract: The first total synthesis of the ramomanin family of lignan natural products is described. The short synthesis involves the intermediacy of a 2,5-diaryl-3,4-dimethylene tetrahydrofuran, which participates in an unexpectedly facile Diels–Alder dimerization, generating all four natural products. Insights into the reactivity and stereoselectivity of the key dimerization are provided through computational studies employing B3LYP/6-31G(d) and M06-2X/6-31G(d) model chemistries.

Guaiacum officinale and *Guaiacum sanctum* are the sources of the highly prized lignum vitae wood, known for its extraordinary strength and durability. The resin of lignum vitae, known as gum guaiac, or guaiac resin, is also valuable. It is reported to possess various medicinal properties,[1] and has also found application in colorimetric tests for oxidative conditions (*e.g.*, the "Nobles' Test",[2] the "Guaiac Occult-Blood Test"[3] and the "Schönbein–Pagenstecher Test"[4]). α-Guaiaconic acid (**1**) is the constituent of gum guaiac that is readily oxidized, forming the vividly colored guaiacum blue (**2**) (Scheme 1a).[5] Sadly, the highly sought-after attributes of lignum vitae wood and gum guaiac have led to overexploitation of *Guaiacum officinale*, which is now listed on the IUCN (International Union for the Conservation of Nature) Red List as an endangered species.[6]

The ramonanins A–D (**3–6**) are a group of spirocyclic phenylpropanoid tetramers isolated from the heartwood of *Guaiacum officinale* by Schroeder and co-workers in 2011 (Scheme 1b).[7] The ramonanins (**3–6**) formulate as dimers of α-guaiaconic acid (**1**) and are reported to exhibit cytotoxic activity against human breast cancer cell lines.[7] The endangered status of *Guaiacum officinale,* coupled with promising biological activity of its natural products, are classic motivations for total synthesis efforts.[8] In truth, however, our attention was drawn to these molecules because of their intriguing structures, which are fascinating from both a topological and biosynthetic perspective. The spirocyclic core of the ramonanins is unprecedented among natural products and Schroeder proposed a biosynthetic pathway that involved dimerization of a lignan precursor, **7**, via a *"Diels–Alder-like mechanism"* (Scheme 1b).[7]

Schroeder's proposed biosynthetic intermediate **7** presumably also serves as the biosynthetic precursor to α-guaiaconic acid (**1**). Since there are no reports of 1,2-dimethylenecyclopentane-*type* structures undergoing Diels–Alder dimerizations under ambient conditions,[9] it seems reasonable to assume that lignan **7** would not undergo spontaneous Diels–Alder dimerization.[10] Our previous

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Scheme 1. (a) The structure and reactivity of α-guaiaconic acid (**1**) (b) Schroeder's proposed biosynthesis of the ramonanins A–D (**3–6**).[7]
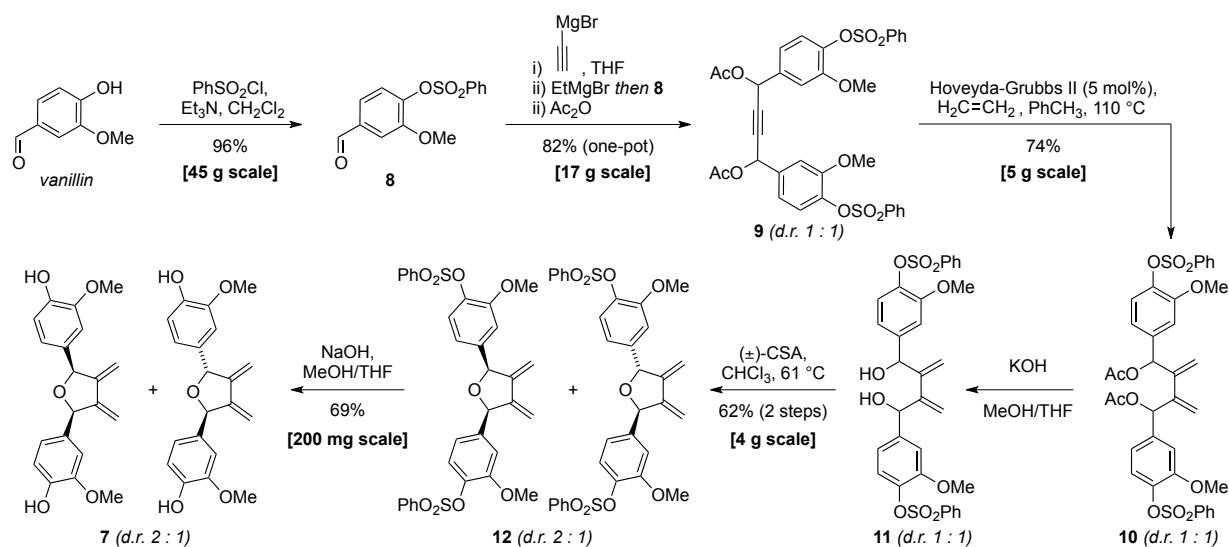
Scheme 2. Synthesis of lignan **7**.

studies on the kingianin natural products utilized a radical-cation catalyzed formal Diels–Alder (RCDA) reaction to dimerize a thermally unreactive bicyclo[4.2.0]octadiene structure.[11] We considered that the ramonanins A–D (**3**–**6**) might also be the result of a biosynthetic RCDA dimerization.[12] In order to investigate the nature of this fascinating biosynthetic dimerization we elected to pursue a total synthesis of the ramonanin natural products (**3**–**6**).[13]

The synthesis began with the high yielding conversion of vanillin into its benzene sulfonate ester, a process that could be easily conducted on large scale (Scheme 2). Two molecules of the resulting aldehyde **8** were united through an acetylene unit in a one-pot sequence to afford multi-gram quantities of diacetate **9**, as a 1:1 mixture of diastereomers. Following a strategy pioneered by Mori and co-workers,[14] alkyne **9** was subjected to an enyne metathesis with ethylene using the Hoveyda–Grubbs II catalyst, to afford diene **10** on multigram scale. Deprotection of the secondary alcohols was accomplished using KOH in MeOH to afford diol **11**. Camphor sulfonic acid was then used to promote 3,4-dimethylene tetrahydrofuran ring formation, which generated protected lignan **12** as a 2:1 mixture of diastereomers, both of which would be required for the synthesis of ramonanins A–D (**3**–**6**). This short synthetic sequence allowed access to multi-gram quantities of key intermediate **12**, which could be stored at −15 °C for weeks without deterioration. When required, the benzene sulfonate protecting groups could be cleanly removed, using NaOH in MeOH/THF, to afford lignan **7** (Scheme 2).

To our surprise and delight, dimerization of lignan **7** was observed to occur spontaneously at room temperature, albeit slowly.[15] Under optimized conditions, dissolution of monomer *cis*-**7**[16] in a minimal amount of DMF and warming at 50 °C provided, after chromatographic purification, a 54:9:37 mixture of ramonanins A (**3**), B (**4**) and C (**5**) in a combined 45% yield, in addition to 24% recovered starting material *cis*-**7** (Scheme 3).[17] Preparative HPLC allowed the isolation of analytically pure samples of ramonanins A−C (**3**–**5**) and the spectroscopic data for each synthetic compound matched those reported for the corresponding natural product, thus securing the total synthesis of ramonanins A−C (**3**–**5**). Optical rotations for the natural products were reported in the isolation paper (**3**: $[\alpha]_D^{22}$ −2.2 (*c* 0.6, MeOH); **4**: $[\alpha]_D^{22}$ −4.0 (*c* 0.2, MeOH); **5**: $[\alpha]_D^{22}$ −5.2 (*c* 0.2, MeOH); **6**: $[\alpha]_D^{22}$ −5.1 (*c* 0.3, MeOH)).[7] Nevertheless, since ramonanins A−C are the Diels–Alder dimers of achiral diene *cis*-**7**, we strongly suspected that these natural products were racemates. To test this hypothesis, we performed chiral HPLC analysis on samples of ramonanins A and C, kindly provided by Professor Schroeder,[18] which confirmed their racemic nature.
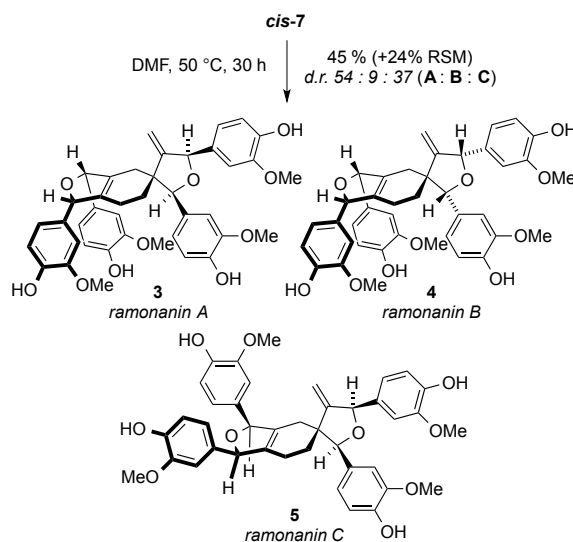
Scheme 3. Dimerization of lignan *cis*-**7**.

Our attention now turned to the final member of the family: ramonanin D (**6**) (Scheme 1). A unique stereochemical feature of ramonanin D (**6**) is the *trans* relationship between the two aryl substituents on the dihydrofuran ring. As would be expected, upon warming a mixture of *cis*-**7** and *trans*-**7**, complex mixtures of products were obtained. Nevertheless, an analytically pure sample of ramonanin D (**6**) was isolated following multiple HPLC purifications, albeit in low (<1%) yield.

Given the surprisingly facile nature of the Diels–Alder dimerization of *cis*-**7**, we elected to investigate the reactivity and stereochemical control of this process computationally, using the B3LYP/6-31G(d) and M06-2X/6-31G(d) model chemistries. The 2,5-diphenyl analogue of *cis*-**7**, namely **13** (Scheme 4), was used as a simulacrum.[19] Eight distinct stereochemical modes of DA dimerization, and hence eight transition structures (TSs), are possible, and these are depicted in Scheme 4. The TSs are described using notation where the Alder-Stein mode may be either *endo* (*N*)

or *exo* (***X***) and the pair of phenyl substituents on the diene and on the dienophile may be either *anti* (***a***) or *syn* (***s***), with respect to the bond forming zone. The eight TSs comprise four pairs, both members of each pair giving one of the ramonanin simulacra adducts (***R$_X$*** = simulacra of ramonanin X; ***R$_{NOE}$*** = not observed experimentally).
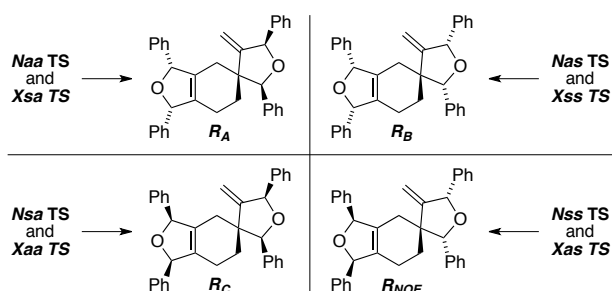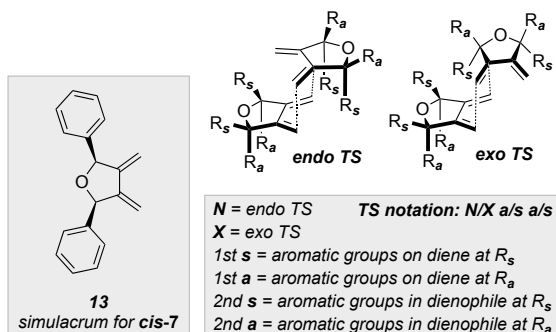
Scheme 4. The eight stereoisomeric TSs for the dimerization of **13** to afford the ramonanin simulacra (***R$_A$***, ***R$_B$***, ***R$_C$*** and ***R$_{NOE}$***), with explanation of the TS notation.

Table 1. B3LYP/6-31G(d) and experimental product distributions (%) for the DA dimerization of *13* and *cis*-**7**, respectively.

Adduct	Mode	Predicted	Total Predicted	Expt
R$_A$	*Naa*	58.6	59	54
	Xsa	0.1		
R$_B$	*Nas*	3.7	4	9
	Xss	0		
R$_C$	*Nsa*	17.4	35	37
	Xaa	17.8		
R$_{NOE}$	*Nss*	0.1	2	-
	Xas	2.3		

The computed product distribution for the Diels–Alder dimerization of **13** is presented in Table 1, together with the experimentally determined product distribution from dimerization of *cis*-**7** (Scheme 3). The agreement between the predicted and experimental adduct distribution is good and strongly suggests that, not only is the model chemistry used in this study reliable, but also that the –OH and –OMe substituents in *cis*-**7** do not significantly affect the distribution of the ramonanin stereoisomers. As expected, the ***Naa*** and ***Xaa*** TSs are the most favorable, presumably because both pairs of phenyl groups (*i.e.*, from diene and dienophile) occupy the *anti* space with respect to the bond forming zone, with the former lying 3 kJ/mol below the latter, in accordance with Alder's *endo* rule. TSs ***Nss*** and ***Xss***, with all phenyls positioned in the *syn* space, lie, respectively, 16 and 24 kJ/mol above the ***Naa*** TS, reflecting steric congestion of the phenyl groups, as may be seen by

comparing the geometries of ***Naa*** and ***Nss*** TSs in Figure 1. All TSs were found to be highly asynchronous, with the shorter forming bond lying between 1.72 and 1.97 Å and the longer between 3.04 and 3.69 Å, the most asynchronous being the ***Nss*** TS (Δr = 1.97 Å, Figure 1).
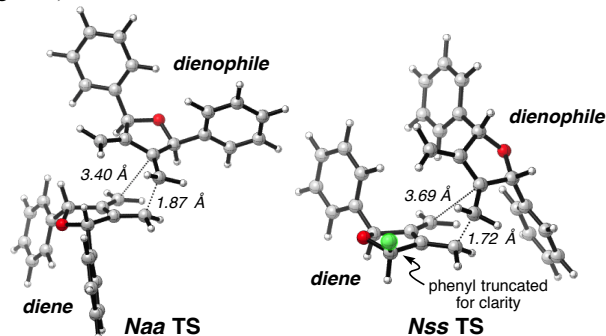

Figure 1. Transition Structures ***Naa*** and ***Nss*** with forming bond lengths.

It seems surprising that neat samples of *cis*-**7** readily dimerize, as does cyclopentadiene (CPD), given that the terminal methylene carbon atoms in **13** are calculated to be 3.139 Å apart, compared to only 2.363 Å in CPD.[20] The M06-2X computed activation enthalpies and free energies for dimerization of CPD, 1,2-dimethylenecyclopentane, 3,4-dimethylenetetrahydrofuran and **13** are listed in Table 2.

Table 2. ΔH‡ and ΔG‡ values (kJ/mol, 25 °C) for DA dimerization of various dienes.[a]

Entry	Reactant	ΔH‡	ΔG‡
1		60.7	121.7
2		64.2	125.0
3		59.8	122.2
4	**13**	58.0	132.2

[a] M06-2X/6-31G(d)-[PCM=THf]//M06-2X/6-31G(d) activation energies from the lowest energy (*endo*) path.

As may be seen from the Table, ΔH‡ for DA dimerization is approximately the same for all four reactants and, in particular, neither the presence of phenyl groups nor that of the ring oxygen has any significant effect on ΔH‡, *cf.* entries 3 with 4 and 2 with 3, respectively. ΔG‡ for dimerization of **13** is, however, about 10 kJ/mol larger than those for the other three systems, which are similar to each other. This free energy difference translates into a 50-fold decrease in the DA dimerization rate for **13**, compared to the other dienes listed in the Table. The origin of this effect is probably due to the phenyl groups in **13** experiencing a steeper potential for their torsional motion about the bonds connecting them to the tetrahydrofuran ring in the TS, compared to the reactant. In conclusion, these calculations support the notion of a concerted

cycloaddition event, both in the synthesis and biogenesis of the ramonanin natural products.

In summary, a 7 step total synthesis of the ramonanin natural products has been achieved. An investigation of the chemistry of Schroeder's proposed biosynthetic pathway has pinpointed the most likely origin of these fascinating structures. The natural products have been shown to be racemates. Our experimental results demonstrate that lignan **7** is pre-disposed towards thermal Diels–Alder dimerization, and our computational findings predict this behaviour more widely in the 1,2-dimethylene cyclopentane series. It remains unclear whether this pre-disposed reactivity is being harnessed in nature constructively, or is generating products that are artifacts of isolation.

[1]   a) R. S. Munger, *J. Hist. Med. Allied Sci.* **1949**, *IV*, 196–229; b) J. F. Morton, *Atlas of Medicinal Plants of Middle America: Bahamas to Yucatan*, Charles C. Thomas, Springfield, IL, **1981**; c) J. L. Hartwell, *Lloydia* **1971**, *34*, 386–425; d) L. P. Somogyi, *Food Additives*, John Wiley & Sons, Inc., New York, **2000**.

[2]   M. K. Nobles, *Can. J. Bot.* **1958**, *36*, 91–99.

[3]   D. C. Rockey, *N. Engl. J. Med.* **1999**, *341*, 38–46.

[4]   W. Autenrieth, *Laboratory manual for the detection of poisons and powerful drugs*, 4th ed. (Ed.: W. H. Warren), Blakiston, Philadelphia, **1915**, 21–22.

[5]   The structure of guaiacum blue (**2**) in Scheme 1a is that shown in the following publication, but it should be noted that this compound has never been fully characterized, see: J. F. Kratochvil, R. H. Burris, M. K. Seikel, J. M. Harkin, *Phytochemistry* **1971**, *10*, 2529–2531.

[6]   *"Guaiacum officinale L."* Taxonomic Serial No.: 897104 Retrieved [07-18-2014], from the ITIS (Integrated Taxonomic Information System) on-line database, http://www.itis.gov. Listed as endangered on the IUCN (International Union for the Conservation of Nature) Red List. Retrieved [07-18-2014], from http://www.iucnredlist.org.

[7]   K. J. Chavez, X. Feng, J. A. Flanders, E. Rodriguez, F. C. Schroeder, *J. Nat. Prod.* **2011**, *74*, 1293–1297.

[8]   For a discussion of the role of total synthesis in addressing the problem of natural product supply, see J. D. Keasling, A. Mendoza, P. S. Baran, *Nature* **2012**, *492*, 188–189.

[9]   This lack of literature precedent perhaps explains why Schroeder and co-workers described the key step in their proposed biosynthesis as

having a *"Diels–Alder-like mechanism"* (Scheme 1). The most closely related example of this type of Diels–Alder reaction comes from Bloomquist and coworkers who, in 1956, synthesized 1,2-dimethylenecyclopentane and reported that attempts to distil the product at 97 °C resulted in dimerization, see: A. T. Blomquist, J. Wolinsky, Y. C. Meinwald, D. T. Longone, *J. Am. Chem. Soc.* **1956**, *78*, 6057–6063.

[10] For an excellent review on biosynthetic Diels–Alder reactions, see: E. M. Stocking, R. M. Williams, *Angew. Chem.* **2003**, *115*, 3186–3223; *Angew. Chem. Int. Ed.* **2003**, *42*, 3078–3115.

[11] S. L. Drew, A. L. Lawrence, M. S. Sherburn, *Angew. Chem.* **2013**, *125*, 4315–4318; *Angew. Chem. Int. Ed.* **2013**, *52*, 4221–4224.

[12] Relatively few examples of total syntheses containing proposed biomimetic radical-cation Diels–Alder reactions have been reported, see Ref [11] and: a) H. Cong, C. F. Becker, S. J. Elliot, M. W. Grinstaff, J. A. Porco, *J. Am. Chem Soc.* **2010**, *132*, 7514 –7518; b) S. Lin, M. A. Ischay, C. G. Fry, T. P. Yoon, *J. Am. Chem. Soc.* **2011**, *133*, 19350–19353; c) H. N. Lim, K. A. Parker, *Org. Lett.* **2013**, *15*, 398–401; d) C. Qi, H. Cong, K. J. Cahill, P. Müller, R. P. Johnson, J. A. Porco, *Angew. Chem. Int. Ed.* **2013**, *52*, 8345–8348; e) H. N. Lim, K. A. Parker, *J. Org. Chem.* **2014**, *79*, 919–926.

[13] For our previous biomimetic studies on dimeric natural products, see Ref [11] and: a) P. D. Brown, A. C. Willis, M. S. Sherburn, A. L. Lawrence, *Angew. Chem.* **2013**, *125*, 13515–13517; *Angew. Chem. Int. Ed.* **2013**, *52*, 13273–13275; b) P. D. Brown, A. C. Willis, M. S. Sherburn, A. L. Lawrence, *Org. Lett.* **2012**, *14*, 4537–4539.

[14] Select examples: a) A. Kinoshita, N. Sakakibara, M. Mori, *J. Am. Chem. Soc.* **1997**, *119*, 12388–12389; b) A. Kinoshita, N. Sakakibara, M. Mori, *J. Am. Chem. Soc.* **1997**, *119*, 12388–12389; c) K. Tonogaki, M. Mori, *Tetrahedron Lett.* **2002**, *43*, 2235–2238; d) M. Mori, K. Tonogaki, N. Nishiguchi, *J. Org. Chem.* **2002**, *67*, 224–226.

[15] The half-lives of both diastereomers of lignan **7** were determined to be approximately 2 days, for mixtures of diastereomers dissolved in a minimum of DMF (14.6 M) at 25 °C; see the Supporting Information for details.

[16] The *cis* and *trans*-diastereomers of compound **12** could be separated via preparative HPLC and then used to prepare pure samples of *cis*-**7** and *trans*-**7**, see the Supporting Information for details.

[17] The reaction was halted before completion to avoid the formation of decomposition products. Attempts to bring about a RCDA reaction using aminium radical catalysis (D. A. Belville, D. D. Wirth, N. L. Bauld, *J. Am. Chem. Soc,* **1981**, *103*, 718–720), or photoredox catalysis (S. Lin, M. A. Ischay, C. G. Fry, T. P. Yoon, *J. Am. Chem. Soc.* **2011**, *133*, 19350–19353), were met with failure.

[18] An authentic sample of ramonanin B was not available.

[19] Full details of these calculations are given in the Supporting Information.

[20] The fast Diels–Alder dimerization of cyclopentadiene has been ascribed to its short C1–C4 distance, see: R. Sustmann, M. Böhm, J. Sauer, *Chem. Ber.* **1979**, *112*, 883–889.
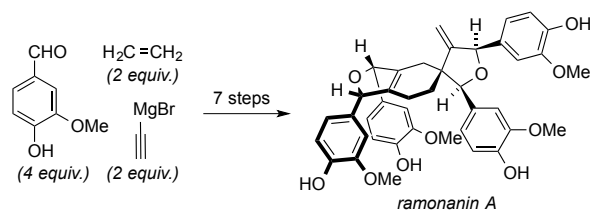
ramonanin A

Dimers of dimers: The first total synthesis of the ramonanin family of natural products has been achieved in short order. These natural phenylpropanoid tetramers were assembled in 7 steps from the starting materials vanillin, ethylene and the ethynyl Grignard reagent. Computational studies shed light on a surprisingly facile Diels–Alder dimerization.