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Synthesis and Biological Evaluation of Himanimide C and Unnatural Analogues

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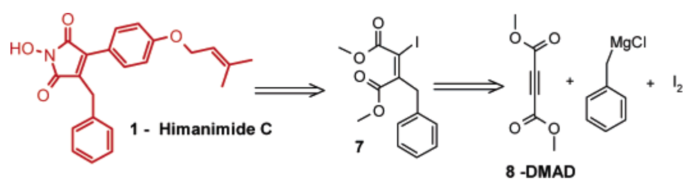
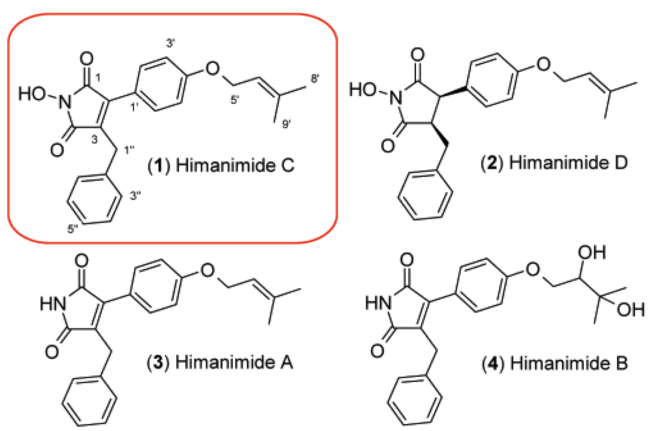
ABSTRACT



Recently isolated himanimide C (1) can be prepared via a short, flexible, and stereoselective synthesis using a copper-mediated tandem vicinal difunctionalization of dimethyl acetylenedicarboxylate (DMAD, 8) as a key step. The flexibility of the synthesis is exemplified by the preparation of new unnatural himanimide analogues in order to investigate the fungicidal potency of this new family.

Himanimides **1**−**4** were recently isolated from basidomycete culture in Chile and were fully characterized by spectroscopic methods as new maleimide derivatives which inhibit the growth of bacteria and fungi.[1] Himanimides were tested against filamentous fungi, yeast, and bacteria as well as different cell lines in order to evaluate potential biological activity. Himanimide C (**1**) exhibits good to excellent antimicrobial activity,[2] and the authors suggested that it could be linked to the N-hydroxylated maleimide moiety. As we believe this original N-hydroxylated maleimide moiety could be important for biological activity, it appeared to us that natural compounds with such simple structures could be interesting leads in the search for new antifungal agents for plant pathogens. Herein, a short and flexible synthesis of himanimide C and related compounds is presented.

To evaluate rapidly structure−activity relationships around the scaffold of himanimides, we required a flexible approach that would allow us to investigate the benzylic and the aromatic region of the molecule as well as the N-hydroxylated maleimide moiety.



Our disconnection strategy is described in Scheme 1 for himanimide C and analogues. The introduction of the hydroxylated amide can be envisaged at the last step from either diesters **5** or the corresponding maleic anhydride.

Preparation of tetrasubstituted olefins **5** can be envisaged by cross-coupling boronic acid **6** and iodo diesters **7**. The desired iodo diesters **7** could be synthesized via copper-mediated tandem vicinal difunctionalization of DMAD **8**.[3,4] Boronic acids **6** can be prepared from readily available 4-bromophenol derivatives.

(1) Aqueveque, P.; Anke, T.; Sterner, O. *Z. Naturforsch.* **2002**, *57c*, 257−262.

(2) At 25 μg/mL, himanimide C exhibits a fungicidal activity against *Alternaria porri*, *Aspergillus ochraceus*, and *Pythium irregulare*.

(3) Ratemi, E. S.; Dolence, J. M.; Poulter, C. D.; Veradas, J. C. *J. Org. Chem.* **1996**, *61*, 6296−6301

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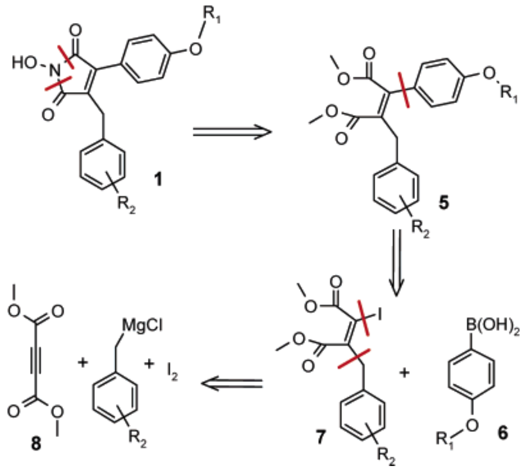
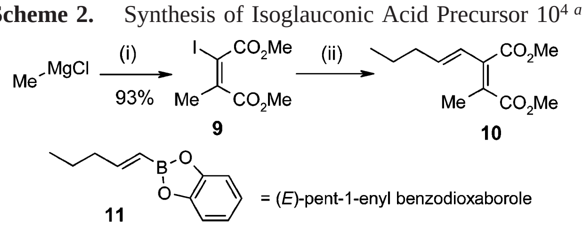
Scheme 1. Disconnection Strategy



Our first objective was to prepare stereoselectively the tetrasubstituted alkenes **7** from DMAD and the organocopper derived from benzylic Grignard reagents. Such a methodology has been reported for the synthesis of natural occurring maleic anhydrides such as chaetomellic anhydride A[3] and isoglaucanic acid (Scheme 2).[4]

Scheme 2. Synthesis of Isoglauconic Acid Precursor 10[4] [a]

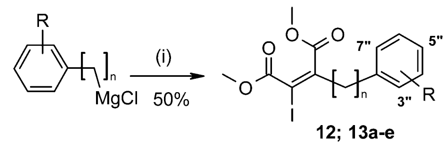


[a] Reagents and conditions: (i) Grignard reagent added to CuBr·Me_2S, THF, −40 °C, 30 min then DMAD, THF −78 °C 40 min then iodine in THF added in 30 min and 90 min at −78 °C; (ii) $Pd(OAc)_2$, PPh_3, K_2CO_3, EtOH, (*E*)-pent-1-enyl benzodioxaborole **11**.

As shown in Scheme 2, Baldwin et al.[4] prepared the intermediate **9** by reacting an alkyl Grignard with DMAD in the presence of CuBr·Me_2S followed by an in situ quench with iodine. The tetrasubstituted iodoalkene **9** was then converted into **10** by cross coupling with (*E*)-pent-1-enyl benzodioxaborole **11**.

At the outset of our work, the use of benzylmagnesium halides in such a vicinal difunctionalization of acetylenes bearing electron-withdrawing substituents was unknown to our knowledge. In a first attempt, following the Baldwin procedure,[4a] we did not succeed in obtaining the corresponding tetrasubstituted olefin by treating DMAD with benzylmagnesium chloride in the presence of CuBr·Me_2S followed by iodine addition. However, with longer reaction times for the organocuprate formation, and for the conjugated addition, we could obtain the tetrasubstituted vinyl iodide **12** as a single isomer in 50% yield (Table 1).

Table 1. Preparation of Tetrasubstituted Olefins[a]



compd	*n*	substituents	yields[b]
12	1		50
13a	1	5″-fluoro	26
13b	0	5″-phenyl	20
13c	1	3″-methoxy	38
13d	2		54
13e	0	3″,7″-dimethyl	33

[a] Reagents and conditions: (i) magnesium chloride added to CuBr·Me_2S, THF, −40 °C, 2 h then DMAD, THF −78 to −40 °C, 2 h, and quenched with iodine in THF −40 °C to rt. [b] Isolated yields (%).

Our observation suggests that the benzylic organometallic species are less reactive than the corresponding alkyl species prepared in the former reports[3−5] where the anionic character cannot be stabilized by the adjacent aromatic nucleus.[6]

To study further the difunctionalization of DMAD and also to investigate the biological importance of the benzyl moiety of himanimides, we repeated the reaction for the preparation of compounds **13a**−**e** (Table 1).[7] It is noteworthy that the best yield was obtained when the homobenzylic magnesium chloride was used as a nonstabilized organometallic intermediate.

With the tetrasubstituted iodo alkenes (**12**; **13a**−**e**) in hand, we next investigated their Suzuki cross-coupling reactions with boronic acids **6**. The commercially available 4-methoxyphenylboronic acid was first used as a model using standard coupling conditions[8] (Table 2).

These model couplings proceeded in good to excellent yield in each instance, providing even the hindered tetrasubstituted olefin **16e** in 82% yield.

At this stage, attempts to cyclize directly the diester **15** following the Chan procedure[9] with hydroxylamine under basic conditions failed to provide the N-hydroxylated male-

(4) (a) Adlington, R. M.; Baldwin, J. E.; Cox, R. J.; Pritchard G. J. *Synlett* **2002**, *5*, 820−822. (b) Baldwin, J. E.; Adlington, R. M.; Roussi, F.; Bulger, P. G.; Marquez, R.; Mayweg, V. W. *Tetrahedron* **2001**, *57*, 7409−7416. (c) Baldwin, J. E.; Beyeler, A.; Cox, R. J.; Keats, C.; Pritchard G. J.; Adlington, R. M.; Watkin, D. J. *Tetrahedron* **1999**, *55*, 7636−7374.

(5) Alexakis, A.; Cahiez, G.; Normant, J. F. *Synthesis* **1979**, 826−830.

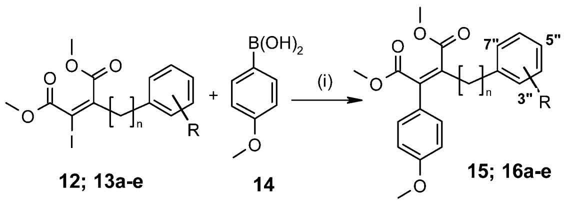
(6) By reacting a 1/1 mixture of butylmagnesium chloride and benzylmagnesium chloride under the conditions described in Table 1, we obtained a 4/1 mixture in favor of the butylalkenes. For a discussion concerning the relative reactivity of Grignard reagents, see: Sonoda, S.; Houchigai, H.; Asaoka, M.; Takei, H. *Tetrahedron Lett.* **1992**, *33* (22), 3145−3146.

(7) All of the compounds prepared for this study were characterized by spectroscopic methods: ^{1}H, ^{13}C NMR, and MS (EI).

(8) (a) Suzuki, A. *Pure Appl. Chem.* **1985**, *57*, 1749−1758. (b) For a typical example of Suzuki cross-coupling reaction of α-iodo α,*β* unsaturated esters, see: Patent WO 9424085 A1, 1994.

(9) Chan, L. C.; Lien, E. J.; Tokes, Z. *J. Med. Chem.* **1987**, *30*, 509−514.

Table 2. Suzuki Cross-Coupling Reaction[a]



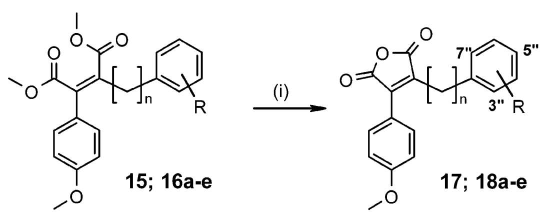
compd	*n*	substituent	yield[b]
15	1		96
16a	1	5″-fluoro	97
16b	0	5″-phenyl	98
16c	1	3″-methoxy	70
16d	2		95
16e	0	3″,7″-dimethyl	82

[a] Reagents and conditions: (i) Pd(TPP)$_4$ (2.5% mol) in toluene /ethanol/ Na_2CO_3 (2 M in water) 3/1/1, reflux (TPP = triphenylphosphine). [b] Isolated yields (%).

imides. Their synthesis via the corresponding maleic anhydrides was therefore investigated.[10] Although this route is less direct, the availability of the maleic anhydrides provides us a means of evaluating the biological significance of the N-hydroxylated moiety.

The anhydride preparations were run under basic conditions followed by an acidic workup.[11] As seen in Table 3, the reaction proceeded from low (**18e**) to good yields (**17**).

Table 3. Saponification−Cyclization[a]



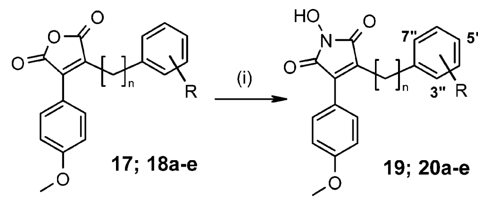
compd	*n*	substituent	yield[b]
17	1		97
18a	1	5″-fluoro	51
18b	0	5″-phenyl	95
18c	1	3″-methoxy	76
18d	2		70
18e	0	3″,7″-dimethyl	27

[a] Reagents and conditions: (i) NaOH 2 N; reflux 2−6 h followed by HCl 1 N, rt. [b] Isolated yields (%).

The lower yield obtained for the cyclization of **16e** is understandable since considerable steric constraints are generated by the formation of the unsaturated five-membered ring (**18e**) possessing the ortho disubstituted phenyl ring adjacent to the second aromatic nucleus. For all the other compounds the presence of at least one methylene group introduces more flexibility and minimizes the steric hindrance between the two aromatic moieties.

We finally investigated the formation of the N-hydroxylated maleimides **19**; **20a**−**e** by treating the previous anhydrides in boiling water with hydroxylamine phosphate. It is noteworthy that under these conditions[12] (Table 4) yields

Table 4. Hydroxylamine Phosphate Treatment[12 a]



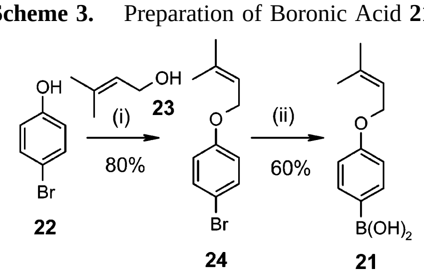
compd	*n*	substituent	yield[b]
19	1		60
20a	1	5″-fluoro	60
20b	0	5″-phenyl	53
20c	1	3″-methoxy	57
20d	2		53
20e	0	3″,7″-dimethyl	52

[a] Reagents and conditions: (i) hydroxylamine phosphate; water reflux, 7 h. [b] Isolated yields (%).

were all in the same range even for the highly hindered **18e** for which we had previously obtained a lower yield during the cyclization.

While we could have prepared Himanimide **1** from compound **19** via a sequence of dealkylation−alkylation of the phenol ring, we decided to use the chemistry described above to introduce the proper aromatic moiety on the iodotetrasubstituted olefin **12**. For this purpose, we prepared the boronic acid **21** as shown in Scheme 3.

Scheme 3. Preparation of Boronic Acid **21**[a]



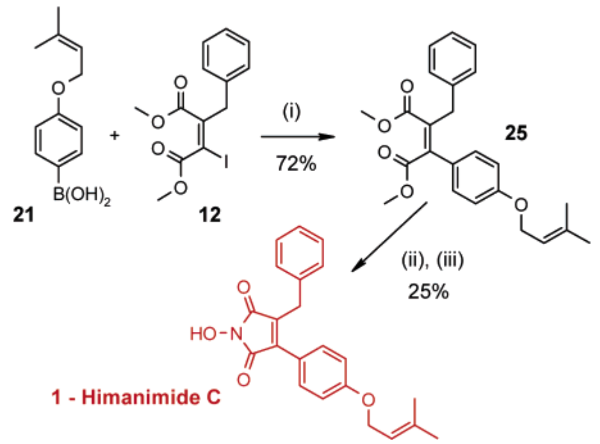
[a] Reagents and conditions: (i) TPP, diisopropyl azodicarboxylate, toluene, rt, 3 h; (ii) (1) *t*-BuLi, THF, −78 °C, (2) B(OMe)$_3$, −78 °C, (3) HCl 1 N, −20 °C.

Commercially available 4-bromophenol **22** was alkylated under Mitsunobu-like conditions[13] with 3-methyl-2-buten-1-ol **23**. Halogen−metal exchange with *t*-BuLi of bromide

(10) Icikawa, Y.; Naganawa, A.; Isobe, M. *Synlett* **1993**, 737−738.
(11) The cyclization reaction can be followed by the color change in the reaction vessels. The anhydrides obtained exhibit in each case a strong fluorescence from yellowish green to reddish yellow. The fluorescence spectra were not registered on these compounds.

24 followed by treatment with trimethyl borate provided after hydrolysis **21** with an overall yield of 48%. With boronic acid **21** in hand, synthesis of himanimide C was realized as described in Scheme 4. The Suzuki cross-coupling reaction

Scheme 4. Synthesis of Himanimide C[a]



[a] Reagents and conditions: (i) Pd(TPP)$_4$ (2.5% mol) in toluene/ethanol/Na_2CO_3 (2 M in water) 3/1/1, reflux 2 h; (ii) NaOH 2 N; reflux 4 h followed by HCl 1 N, rt; (iii) hydroxylamine phosphate; water reflux, 7 h.

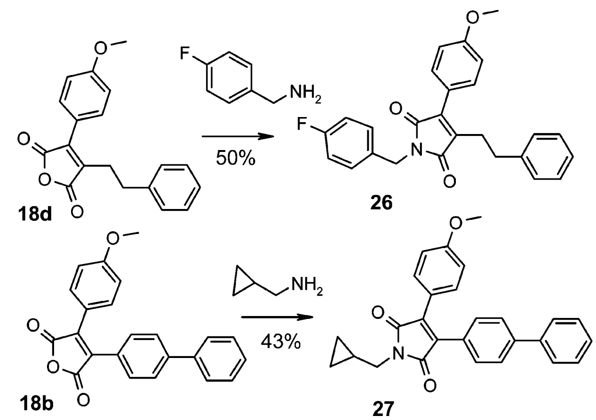
afforded the diester **25**, which was readily transformed by the complete saponification–cyclization–amide formation sequence into the desired himanimide C **1**. The ^{1}H and ^{13}C spectroscopic data obtained for the synthetic himanimide C were identical to those of the natural product, therefore confirming its structure.

The saponification step was low yielding (40%) due to the chemical sensitivity of the unsaturated chain. Nevertheless, applied on gram scale, this synthesis provided us with enough material to run the biological tests.

In addition, we exemplified the flexibility of our approach by preparing the more exotic maleimides **26** and **27** by treating the anhydrides **18b** and **18d** with benzyl- or alkylamines (Scheme 5).

All of the compounds and intermediates were evaluated in our biological screens against the major plant pathogens.[14]

Scheme 5[a]



[a] Reagents and conditions: (i) methanol, reflux, 2–4 h.

In contrast to the previously reported results,[1] we found that none of them exhibited fungicidal activity in vitro or in planta.

Furthermore, we demonstrated that compounds from this family bearing the N-hydroxylated maleimide moiety are rapidly metabolized in our standard biokinetics metabolism assay[15] with a loss of 98% of the parent compound after 24 h of treatment.

In summary, we report a short and flexible synthetic access to himanimide C (**1**). Related natural and nonnatural compounds were successfully prepared via this methodology. We also demonstrated that himanimide C was not active as a fungicide against plant pathogens in our assays, which may be linked to the poor metabolic stability.

Acknowledgment. We are grateful to Dr. John Clough and Dr. Fiona Murphy for fruitful discussions and proposals. We thank Y. Laime for performing technical work and analysis and Dr. Ian Southworth for biokinetic measurements.

Supporting Information Available: Experimental procedures and analytical data for the preparation of himanimide C **1** and all intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

OL047664O

(12) Patent EP 1 085 013 A1, 2000.

(13) Sankara, S. R.; Balasubramanian, K. K. *Synth. Commun.* **1989**, *19* (7–8), 1255–1259.

(14) The compounds were tested in vitro (Agar plates) and in planta (leaf disks assays) against Oomycetes (*Plasmopara viticola; Phytophthora infestans*), Ascomycetes (*Pyrenophora teres, Erysiphe graminis*) and Basidiomycetes (*Puccinia recondita, Rhizoctonia solani*)

(15) Biokinetic data were recorded by measuring over a period of time the disappearance of the parent compound in maize cell cultures.