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Total Synthesis and Structural Revision of Clavilactone D

Ken-ichi Takao,* Ryuichi Nemoto, Kento Mori, Ayumi Namba, Keisuke Yoshida, and Akihiro Ogura

Abstract: A structural revision of clavilactone D, a potent inhibitor of protein tyrosine kinases, was achieved by total syntheses of two newly proposed structures. The syntheses relied on ring-opening/ring-closing metathesis, which transformed a cyclobutenecarboxylate into a γ-butenolide. The syntheses confirmed that the correct structure of clavilactone D has an amino group at C-3 instead of a hydroxy group at C-2 in the originally proposed structure.

Clavilactone D is a potent inhibitor of protein tyrosine kinases,[1,2] but its structure has remained unsolved. First, antifungal and antibacterial clavilactones A–C (**1**–**3**) were discovered from cultures of the Basidiomycetous fungus *Clitocybe clavipes* by Arnone and co-workers in 1994 (Figure 1A).[3] Their structures were determined as having a 10-membered carbocycle connected to a hydroquinone or benzoquinone and an α,β-epoxy-γ-lactone. In 2000, Merlini and co-workers isolated related compounds, clavilactones D and E (**5**), from the same fungus by using different culture conditions.[1] Because the NMR spectra of clavilactone D and **2** were similar, the structure of clavilactone D was originally assigned as **4** substituted with a hydroxy group in the quinone ring of **2**. The position of the hydroxy group was elucidated by the HMBC spectrum. Studies of the biological activity of **1**, **2**, and clavilactone D showed inhibitory activity against epidermal growth factor tyrosine kinases,[2] and clavilactone D was the most potent inhibitor (IC_{50} 5.5 μM). The unique structures of clavilactones coupled with their important biological activities have inspired several synthetic investigations.[4,5] Barrett and co-workers achieved the first total synthesis of (+)-clavilactone B (the antipode of **2**).[6] Next, we completed the enantioselective total synthesis of the natural enantiomers of **1** and **2**.[7] In collaborative research with a biology group, we showed that synthetic analog **6**, called seco-clavilactone B, is a novel actin polymerization inhibitor and can serve as a bioprobe for clarifying cytoskeletal dynamics.[8] Later, two total syntheses of clavilactones were published by the Li group[9] and the Yoshimitsu group.[10] Notably, Li and colleagues reported that neither the spectroscopic data for synthesized **4** (the originally proposed structure) nor its regioisomer **7** matched those of natural clavilactone D.[9] The correct structure should be revealed to advance both natural product chemistry and biological research. We herein demonstrate the correct structure of clavilactone D to be **9** (Figure 1B) by total syntheses of two newly proposed structures.

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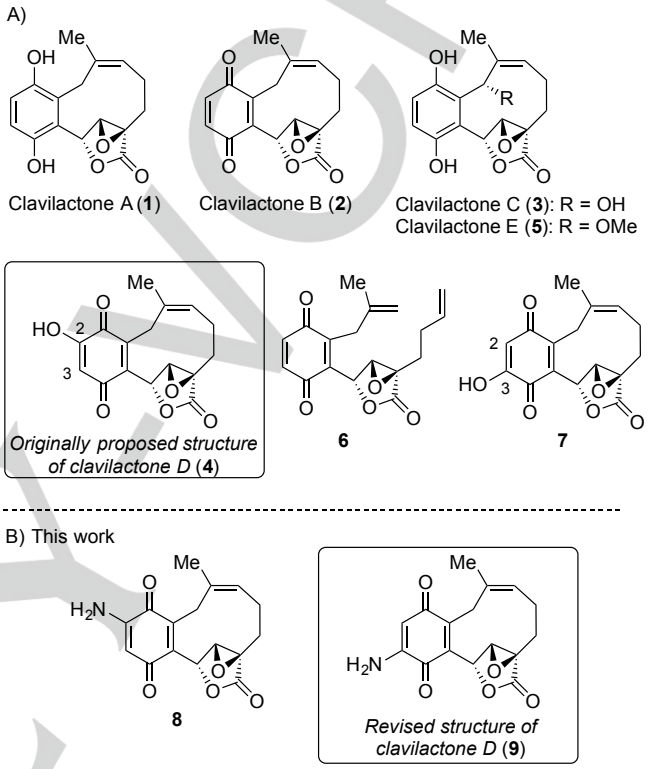
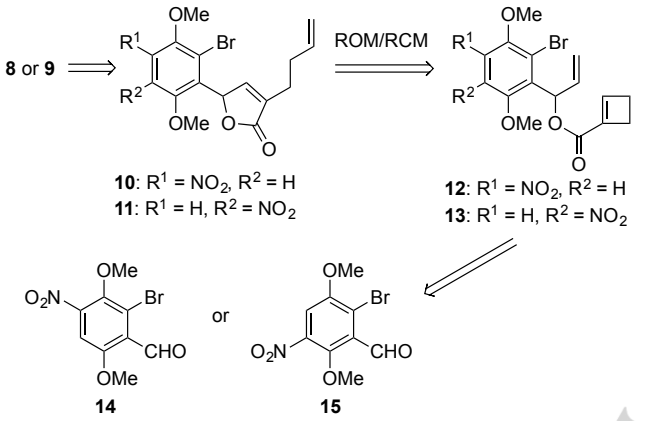


Figure 1. Structures of clavilactones.

Based on the re-evaluation of the published data for clavilactone D,[1] we had doubts about the molecular weight of clavilactone D. In its mass spectrum, the molecular ion was recorded as 302 for M^+ by chemical ionization (CI) MS using CH_4 and no HRMS data was described. The molecular ion in CIMS is usually observed as $[M + H]^+$. Therefore, we assumed that the correct molecular weight of clavilactone D would be 301, one less than the proposed weight. Next, we compared the NMR data of natural clavilactone D with those of **4** and **7** synthesized by Li and colleagues.[9] The signal peak of H-3 (δ 6.15) for **4** or H-2 (δ 6.17) for **7** appeared at a lower field than the corresponding peak (δ 5.90) of natural product. A similar difference was also observed in the ^{13}C NMR spectra (δ 109.2 for C-3 of **4** or δ 109.5 for C-2 of **7** vs. δ 101.7 for natural product). These facts indicate that clavilactone D has a stronger electron-donating group in the quinone ring than the hydroxy group. Consequently, we considered that the revised structure for clavilactone D should be **8** or **9**, containing an amino group at C-2 or C-3, and that the structural revision could be unambiguously confirmed by total syntheses of **8** and **9**.

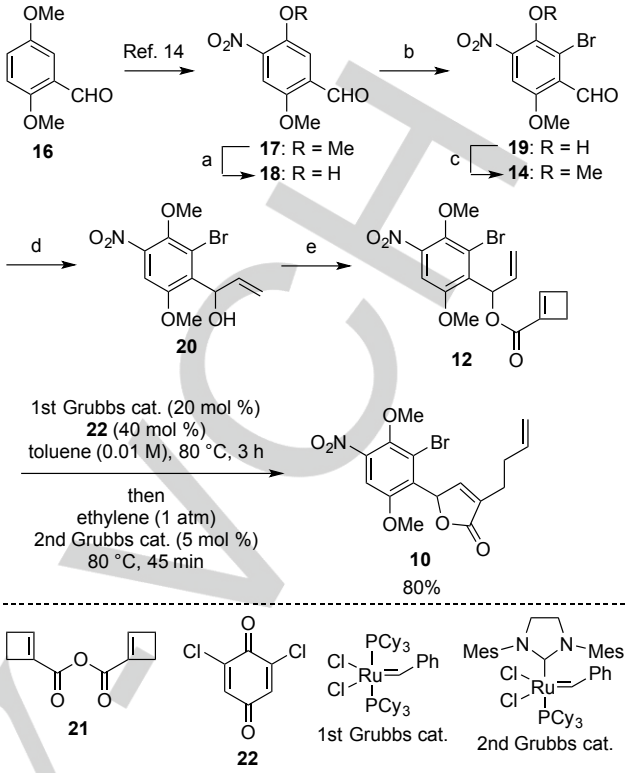
In recent years, we have been interested in ring-rearrangement metathesis, a conceptually new method involving domino metathesis.[11,12] To achieve the total synthesis of **1** and

2, we developed a ring-opening/ring-closing metathesis (ROM/RCM) of cyclobutenecarboxylate derivatives for concise access to γ-butenolides.[4,7] Our strategy has been successfully used for butenolide synthesis by other groups.[13] Therefore, we envisioned an approach that would exploit the ROM/RCM of cyclobutenecarboxylate **12** or **13** to construct γ-butenolide **10** or **11**, which would be transformed into **8** or **9** by a similar sequence of reactions to our previous synthesis of **2** (Scheme 1). The nitro group would be used as a precursor of the amino group at C-2 or C-3. To prepare **12** or **13**, penta-substituted benzene **14** or **15** would be required as a starting material.



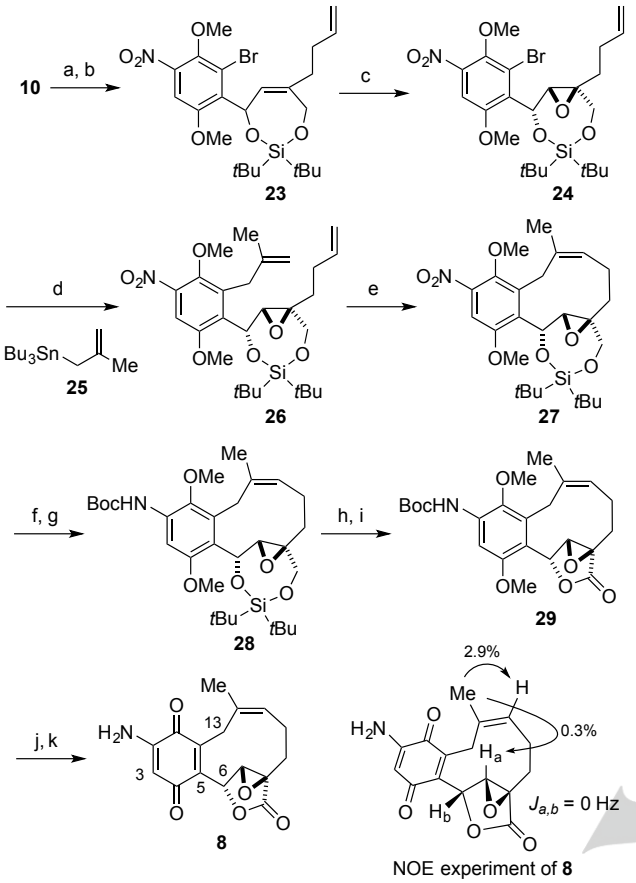
Scheme 1. Retrosynthetic analysis of newly proposed structures **8** and **9** of clavilactone D.

We initially aimed to obtain **8**, which is substituted with an amino group at the same position (C-2) as the hydroxy group in originally proposed structure **4**. The synthesis started with a regioselective preparation of **14** (Scheme 2). According to the known procedure, a nitrated benzaldehyde derivative **17** was regioselectively prepared from 2,5-dimethoxybenzaldehyde (**16**).[14] Despite extensive efforts, corresponding bromide **14** could not be directly obtained from **17**. However, compound **18** underwent a regioselective bromination. Thus, selective demethylation of dimethyl ether **17** with concentrated sulfuric acid[15] provided monomethyl ether **18**.[16] Treatment of **18** with pyridinium tribromide produced desired brominated product **19**, which was re-methylated to **14**. The vinyl Grignard reaction of **14** provided allylic alcohol **20**.[17] Cyclobutenecarboxylate **12** was obtained by acylation of **20** with anhydride **21**.[7] The ROM/RCM reaction of **12** proceeded using the method developed by our group.[7] A solution of the first-generation Grubbs catalyst and benzoquinone **22**[18] was slowly added to a solution of **12** in toluene using a syringe pump. After stirring, the mixture was treated with ethylene and the second-generation Grubbs catalyst to provide γ-butenolide **10** in 80% yield.



Scheme 2. Synthesis of γ-butenolide **10**. Reagents and conditions: a) conc. H_2SO_4, 50 °C, 40 h, 31% (57% based on recovered starting material); b) $PyHBr_3$, MS 4Å, Py, –30 °C, 1 h, 87%; c) MeI, K_2CO_3, DMF, RT, 14 h, 100%; d) CH_2=CHMgBr, THF, –78 °C, 1 h, 73%; e) NaHMDS, **21**, THF, –78 °C, 45 min, 80%. Py = pyridine, MS = molecular sieves, DMF = *N,N*-dimethylformamide, THF = tetrahydrofuran, HMDS = hexamethyldisilazide, Cy = cyclohexyl, Mes = mesityl.

The next stage of the synthesis involved construction of the 10-membered carbocycle of **8** (Scheme 3). Reduction of the γ-lactone in **10**, followed by protection of the resulting diol as a silylene acetal, provided **23**. Chemo- and stereoselective epoxidation of **23** was achieved by using *m*CPBA.[19] Major product **24** was subjected to Stille coupling with allylstannane **25**[20] to afford diene **26**. Ring-closing metathesis (RCM) of **26** with the second-generation Grubbs catalyst proceeded, giving 10-membered carbocycle **27**. The newly formed olefin had the desired geometry.[21] At this stage, the nitro group in **27** was chemoselectively reduced to an amino group under transfer hydrogenation conditions and the resulting product was protected as *t*-butyl carbamate **28**. Removal of the silylene acetal from **28** and subsequent Ley oxidation of the diol reconstructed the γ-lactone, furnishing **29**. Treatment of **29** with CAN, followed by deprotection of the amino group, provided the first target molecule (**8**).



Scheme 3. Synthesis of the first target molecule (**8**). Reagents and conditions: a) DIBAL-H, THF, 0 °C, 2 h; b) tBu$_2$Si(OTf)$_2$, Py, CH_2Cl_2, RT, 30 min, 73% (2 steps); c) *m*CPBA, CH_2Cl_2, phosphate buffer, 0 °C to RT, 4 h, 54% **24**, 16% regioisomer, and 30% di-epoxide (2 cycles); d) **25**, $Pd(PPh_3)_4$, CuCl, 1,4-dioxane, reflux, 12 h, 73%; e) 2nd Grubbs cat. (40 mol %), **22** (60 mol %), toluene (0.7 mM), reflux, 16 h, 74%; f) Pd/C, 1,4-cyclohexadiene, EtOH, 70 °C, 2 h; g) $(Boc)_2O$, Et_3N, 1,4-dioxane, 85 °C, 48 h, 86% (2 steps); h) nBu$_4$NF, THF, RT, 16 h; i) TPAP, NMO, MS 4Å, MeCN, RT, 1 h, 74% (2 steps); j) CAN, MeCN–H_2O, RT, 10 min; k) TFA, CH_2Cl_2, RT, 3.5 h, 54% (2 steps). DIBAL-H = diisobutylaluminum hydride, Tf = trifluoromethanesulfonyl, *m*CPBA = *meta*-chloroperbenzoic acid, Boc = *t*-butoxycarbonyl, TPAP = tetra-*n*-propylammonium perruthenate, NMO = *N*-methylmorpholine oxide, CAN = cerium(IV) ammonium nitrate, TFA = trifluoroacetic acid, NOE = nuclear Overhauser effect.

The NMR data for **8** did not agree with the published natural product data.[1] This included discrepancies in the ^{1}H NMR (e.g., δ 6.13 for H-6 and δ 3.61 for H-13 of **8** vs. δ 5.99 and δ 3.76 for natural product) and the ^{13}C NMR (e.g., δ 144.7 for C-5 of **8** vs. δ 151.3 for natural product). However, compared with **4** and **7**,[9] the difference in the chemical shifts of H-3 (δ 5.86 for **8** vs. δ 5.90 for natural product) and C-3 (δ 149.0 for **8** vs. δ 148.8 for natural product) decreased. In addition, although **4** and **7** were reported as yellow solids by Li and colleagues,[9] compound **8** was obtained as a red solid similar to natural product. Therefore, we moved on to synthesizing the regioisomer of **8**, compound **9**, in which the amino group is substituted at C-3.

This synthesis used known bromide **30**, which was the starting material in our total synthesis of **1** and **2** (Scheme 4).[7] Nitration of **30** proceeded with complete regioselectivity to afford desired penta-substituted benzene **15** as a single isomer.[22] Addition of the vinyl group provided allylic alcohol **31**, which was acylated to furnish key substrate **13** for the ROM/RCM reaction. Cyclobutenecarboxylate **13** was transformed into γ-butenolide **11** by our method. After conversion to silylene acetal **32**, epoxidation followed by Stille coupling of epoxide **33** provided diene **34**. RCM of **34** formed a 10-membered carbocycle to afford **35**, which was converted into **37** through reduction of the nitro group, protection–deprotection, and reconstruction of the γ-lactone. Finally, CAN oxidation followed by deprotection afforded the second target molecule (**9**). The stereochemistry of **9** was confirmed by ^{1}H NMR analysis including NOE experiments. Pleasingly, comparison of the ^{1}H and ^{13}C NMR spectra of synthetic **9** with those of natural clavilactone D showed excellent agreement,[23] indicating that the structure of clavilactone D should be revised to be **9**, and that we had completed the first total synthesis of (±)-clavilactone D. The original ^{1}H NMR spectrum showed a broad signal peak corresponding to –NH_2 at δ 6.59 (δ 6.56 for synthetic **9**), which was assigned as impurities in the paper on the original isolation of clavilactone D. In the original HMBC spectrum, a strong cross peak of the proton in the quinone ring with C-5 ($^4J_{CH}$) was detected, whereas a weak cross peak with C-14 ($^3J_{CH}$) had been missed, leading to the misassignment of the position of the substituent in the quinone ring.

In conclusion, we have synthesized two newly proposed structures, **8** and **9**, of protein tyrosine kinase inhibitor clavilactone D, and we conclude that the true structure of the natural product is **9**, with an amino group at C-3. The key features of the synthesis are regioselective bromination and nitration for preparing penta-substituted benzene derivatives **14** and **15**, an ROM/RCM reaction for transforming cyclobutenecarboxylates **12** and **13** into γ-butenolides **10** and **11** and an RCM for constructing 10-membered carbocycles in **27** and **35**. Our total synthesis has an important role to play in the structural elucidation of clavilactone D,[24] and will enable further biological studies on this class of natural products.

Acknowledgements

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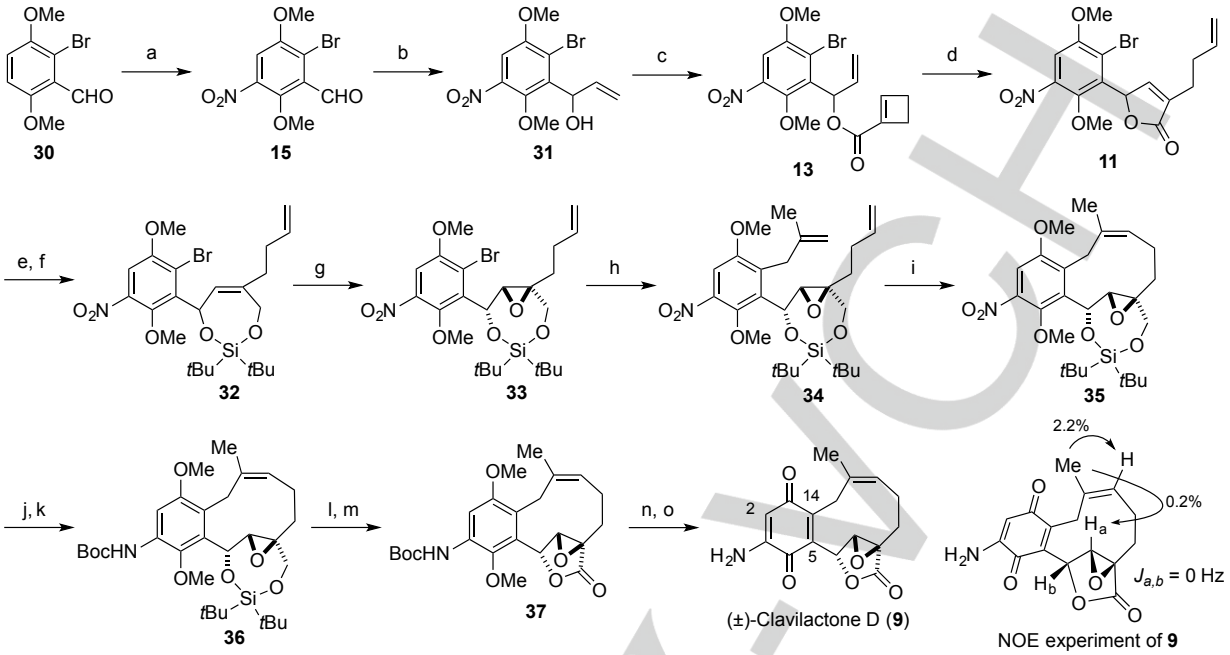
Keywords: medium-ring compounds • metathesis • natural products • structure elucidation • total synthesis



Scheme 4. Total synthesis of (±)-clavilactone D (**9**). Reagents and conditions: a) conc. HNO_3, CH_2Cl_2, RT, 2.5 h, 96%; b) CH_2=CHMgBr, THF, –78 °C, 30 min, 77%; c) NaHMDS, **21**, THF, –78 °C, 1 h, 84%; d) 1st Grubbs cat. (20 mol %), **22** (50 mol %), toluene (0.01 M), 80 °C, 3 h, then ethylene (1 atm), 2nd Grubbs cat. (6 mol %), 80 °C, 40 min, 82%; e) DIBAL-H, THF, 0 °C, 3 h; f) tBu$_2$Si(OTf)$_2$, Py, CH_2Cl_2, RT, 30 min, 65% (2 steps); g) mCPBA, CH_2Cl_2, phosphate buffer, 0 °C to RT, 4 h, 50% **33**, 18% regioisomer, and 31% di-epoxide (2 cycles); h) **25**, $Pd(PPh_3)_4$, CuCl, 1,4-dioxane, reflux, 16 h, 82%; i) 2nd Grubbs cat. (40 mol %), **22** (70 mol %), toluene (0.7 mM), reflux, 16 h, 64%; j) Pd/C, 1,4-cyclohexadiene, EtOH, 70 °C, 1.5 h; k) $(Boc)_2O$, Et_3N, 1,4-dioxane, 85 °C, 64 h, 99% (2 steps); l) nBu$_4$NF THF, RT, 20 h; m) TPAP, NMO, MS 4Å, MeCN, RT, 1 h, 87% (2 steps); n) CAN, MeCN–H_2O, RT, 10 min; o) TFA, CH_2Cl_2, RT 3 h, 65% (2 steps).

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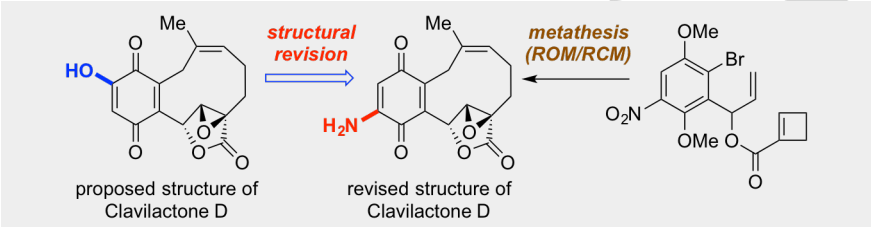

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