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The first total synthesis and neurotrophic activity of clusiparalicoline A, a prenylated and geranylated biaryl from *Clusia paralicola*

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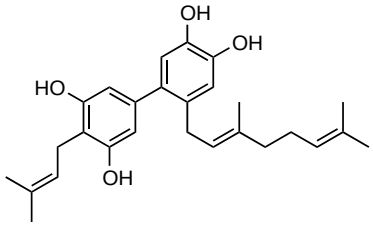
Abstract—The first synthesis of clusiparalicoline A, a prenylated and geranylated biphenyl compound isolated from the roots of *Clusia paralicola*, has been achieved by applying the sequential palladium-catalyzed Stille and Suzuki reactions to the formation of all the C–C bonds on electron-rich aryl bromide and triflate. Clusiparalicoline A has been found to exhibit a potent neurite outgrowth promoting activity at 1.0 μM in a primary culture of fetal rat cortical neurons.

Clusiparalicolines A (**1**) and B (**2**) were isolated as DNA strain-session active compounds from the roots of *Clusia paralicola* and their structures were elucidated by NMR analyses (Fig. 1). They exhibit weak cytotoxicity against the KB human cancer cell.[1] Clusiparalicoline A is a simple biaryl on which the prenyl and geranyl groups are attached. We previously reported that some biphenyl natural products, i.e. mastigophorenes A and B,[2,3] and honokiol,[4] showed intriguing neurotrophic activities on a primary culture of rat cortical neurons. Our interests in evaluating neurotrophic activity of allyl and/or prenyl biphenyl natural products led us to undertake synthesis of **1**. In this letter, we report the first total synthesis of clusiparalicoline A (**1**) by applying sequential palladium-catalyzed cross-coupling reactions and the evaluation of its neurotrophic activity in a primary culture of rat cortical neurons.



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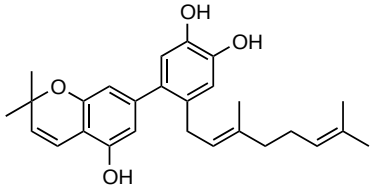

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Figure 1. Structures of clusiparalicolines A (**1**) and B (**2**).

We have envisioned that synthesis of biaryls could be readily accomplished through the Suzuki–Miyaura reaction.[5,6] Because clusiparalicoline A (**1**) is not quite symmetrical, application of such an approach to the synthesis of **1** would require preparation of two geranylated and prenylated phenols bearing different substitution patterns (**3** and **4**). In terms of retrosynthesis, as outlined in Scheme 1, the right half of clusiparalicoline A is readily traced back to a bromophenyl triflate **5**, and assembly of the key intermediate **3** from **5** could be based on palladium-catalyzed Stille[7] and/or Suzuki–Miyaura reactions by utilizing different reactivity of the oxidative addition of aryl bromide and aryl triflate to Pd(0). On the other hand, the left half **4** could be derived from a phloroglucinol **6** by directed *ortho* metalation.[8]

Our synthesis of the right half intermediate began with the commercially available 3,4-dihydroxybenzaldehyde (Scheme 2). After protection of the catechol group of **7**

Keywords: clusiparalicoline A; Stille reaction; Suzuki reaction; palladium; cytotoxicity; neurotrophic activity.

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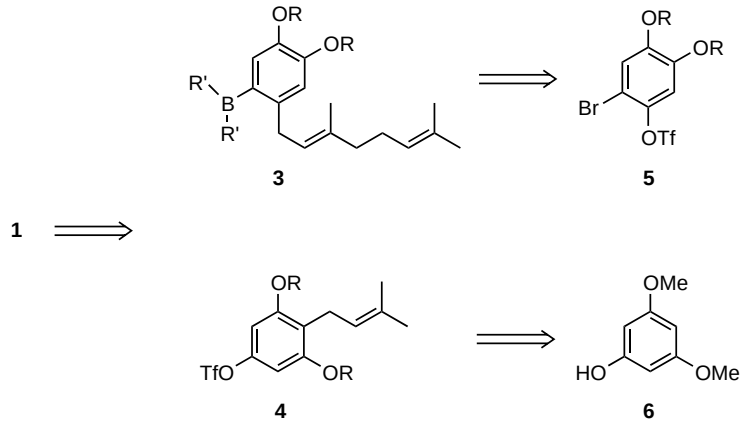
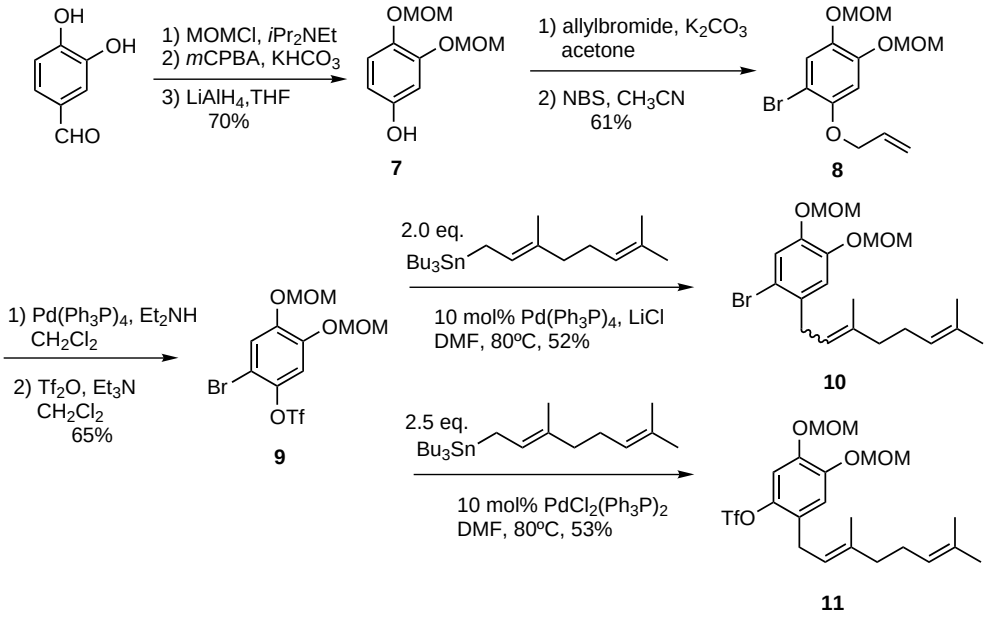


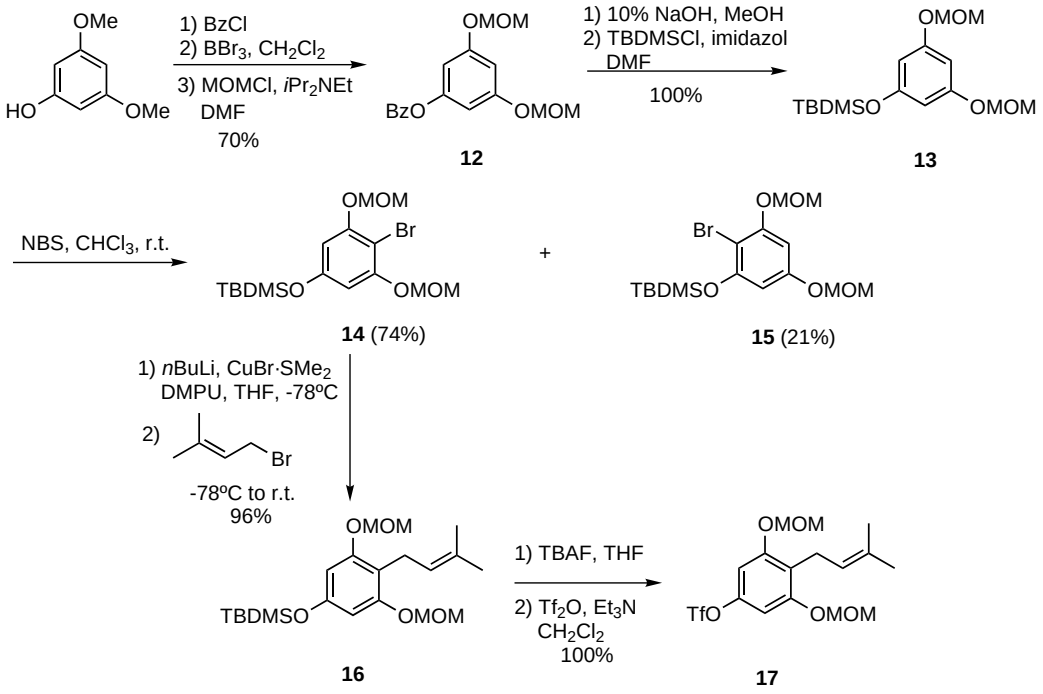
Scheme 1. Synthetic plan of clusiparalicoline A (**1**).



Scheme 2. Preparation of geranylated 2,4,5-trihydroxybenzene derivatives (**10** and **11**).

by reaction with MOMCl and iPr$_2$NEt, Baeyer–Villiger oxidation of the aldehyde, followed by reduction with $LiAlH_4$, afforded **7** in 70% yield. After the formed phenolic group was protected as an allyl ether, a resulting allyl ether was brominated with NBS to give rise to **8** as the sole product in 61% yield.[9] Deallylation of **8** with 15 mol% of $Pd(Ph_3P)_4$ in the presence of an excess of Et_2NH[10] and then reaction with Tf_2O and Et_3N produced **9** which was substituted with a bromine and a triflate group *ortho* to each other in 65% yield over two steps. With the requisite compound **9** in hand, we concentrated on finding out the palladium-catalyzed cross-coupling conditions leading to the right half intermediate appended with a geranyl group. Thus, we attempted the Stille reaction of **9** with geranyl tributyltin[11] under various conditions. After extensive optimization, we found that chemoselective cross-coupling (C–Br versus C–OTf)[12] of **9** with geranyl tributyltin relied on combination of the Pd catalyst, solvent and additive. When **9** was reacted with $Pd(Ph_3P)_4$ (10 mol%), geranyl tributyltin (2.0 equiv.) and LiCl (3 equiv.)[13] in DMF at 80°C for 20 h, the Stille reaction took place on the C–OTf bond, giving rise to the desired intermediate **10** in 52% as a E/Z mixture (2:1).[14] On the other hand, a combination of $PdCl_2(Ph_3P)_2$ (10 mol%), geranyl tributyltin (2.5 equiv.) without an additive such as TBAC and LiCl in DMF at 80°C for 2 h brought about the cross-coupling reaction on the C–Br bond to yield the other desired coupling compound **11** as the sole product in 53% yield.[15]

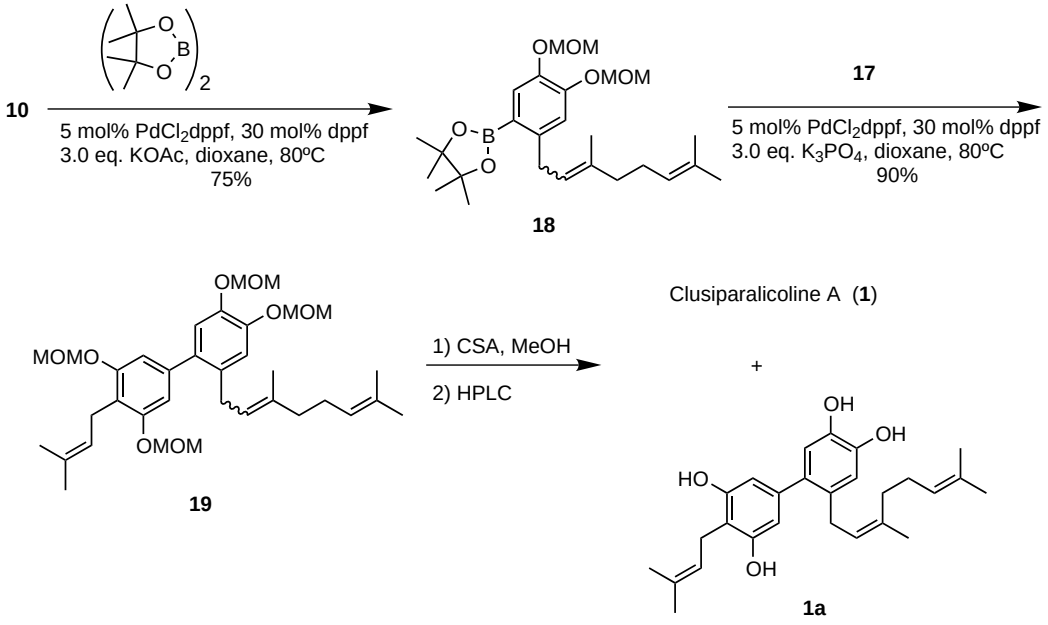
Preparation of the left half intermediate was started from *O*-dimethylphloroglusinol and MOM derivative **13** was readily derived in 70% yield over five steps by the process, as shown in Scheme 3. While the directed *ortho* lithiation of **13** failed to introduce a prenyl group on the position *ortho* to the both MOM groups, alkylation on this particular position was carried out through a bromine–metal exchange. At first, compound **13** was brominated with NBS in $CHCl_3$[17] to afford the desired product **14** and its regioisomer **15** in 74 and 21% yield, respectively. Treatment of **14** with n-BuLi in the pres-



Scheme 3. Preparation of prenylated 2,4,6-trihydroxybenzene derivative (**17**).

ence of $CuBr{\cdot}SMe_2$ at −78°C,[18] followed by adding prenyl bromide, afforded the desired *C*-alkylation product **16** in 96% yield. Removal of the TBDMS and subsequent triflation of **16** produced the left half intermediate **17** with a triflate group quantitatively (Scheme 3).

Now, we are in a position to apply Suzuki–Miyaura reaction to the cross-coupling between the right half unit **10** or **11** and the left half unit **17** (Scheme 4). The Suzuki–Miyaura protocol[19] for direct introduction of a boronic ester to the triflate **11** using bis(pinacolate)diborane were not effective. However, this protocol could provide direct access to a pinacol boronic ester from the bromide **10** using $PdCl_2$dppf (5 mol%), dppf (30 mol%), KOAc (3.0 equiv.) in the presence of bis(pinacolate)diborane when dioxane was used as solvent, resulting in the formation of the boronic ester **18** in 75% yield. Under conditions similar to the preparation of **18**, next Suzuki coupling between **17** and **18** proceed smoothly to afford the coupling product **19** in high yield.[20] Finally, all MOM groups were hydrolyzed



Scheme 4. Synthesis of clusiparalicoline A (**1**).

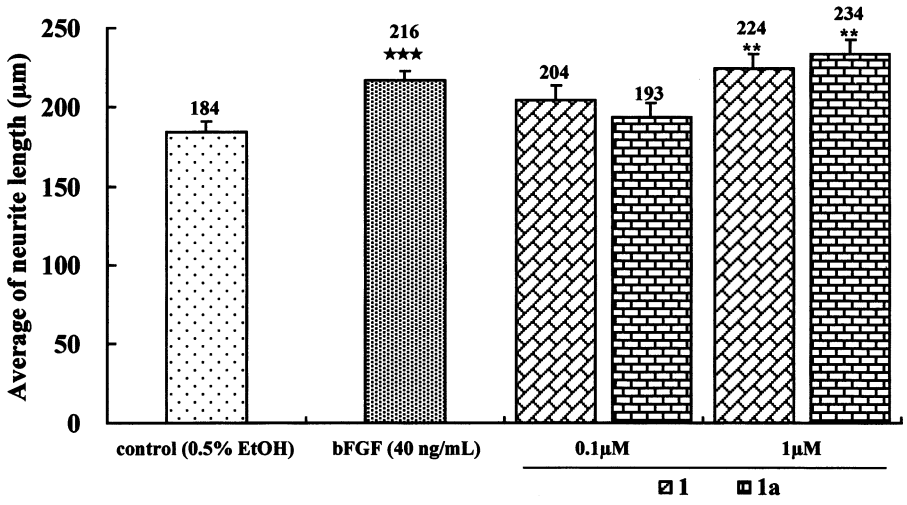


Figure 2. Morphometric analysis of the neurons effected by **1** and **1a**. After the neuronal cells (9000 cells cm^{-2}) cultured for 6 days in the presence of 0.5% EtOH, bFGF, **1**, and **1a** were fixed by 4% paraformaldehyde–PBS, the immunohistochemical staining for MAP-2 was performed. Morphometric analysis was carried out on these neurons according to the criteria.[4] The data are expressed as means±SE ($n=80$); Student's test; ★★★P <0.005 versus control: Dunnet's test; **P <0.01 versus control.

simultaneously by a catalytic amount of CSA[21] in MeOH at room temperature giving rise to a mixture of clusiparalicoline A (**1**) and its *Z* isomer **1a** in 68% yield. Each of the isomers could be readily separated by HPLC.[22] The ^{1}H and ^{13}C NMR of **1** are superimposed with those of natural one. Thus, the first total synthesis of clusiparalicoline A has been accomplished by sequential palladium-catalyzed Stille and Suzuki–Miyaura reactions.

Search for non-peptidal small molecules with neurotrophic properties is of great significance because neurotrophic factors such as the nerve growth factor (NGF) must overcome intrinsic drawbacks associated with polypetidal factors.[23] As stated in introductory remarks, our studies on neurotrophic natural products implies that some biphenyl natural products with allyl and alkenyl substituents may have neurotrophic property.[24] Hence, we examined how clusiparalicoline A (**1**) and its *Z* isomer **1a** effect on neurite outgrowth in a primary culture of rat cortical neurons.[4] As being anticipated, compounds **1** and **1a** have been found to promote neurite outgrowth at 1 μM, as shown in Fig. 2. Their activities are likely to be comparable with bFGF[25] at this concentration. When 10 μM of **1** or **1a** was applied to the neuronal cultures, however, all neurons were killed during 6 day culture period. This result is consistent with the concentration (EC_{50} 8.6 μM) at which clusiparalicoline A exhibits KB inhibitory activity.[1] On the other hand, **1** and **1a** have no effect on morphology and viability of cortical neurons at a lower concentration of 0.1 μM. Wall et al.[1] indicated that the presence of a catechol moiety and a long chain alkyl group such as a geranyl group on the benzene ring were responsible for cytotoxicty but the biaryl moiety was not essential. It was also reported that some biaryls had specific affinity to neuronal cells.[26] Thus, the biaryl moiety bearing alkenyl groups in clusiparalicoline A may have some positive effect on development and survival of neurons, but neurite outgrowth promoting activity caused by clusiparalicoline A may not be closely related to cytotoxicity.

We have accomplished the first synthesis of clusiparalicoline A (**1**) by applying sequential palladium cross-coupling reactions to all the C–C bond formations on the electron-rich aryl bromide and triflate, and have found that **1** and its *Z* isomer **1a** have neurite outgrowth promoting activity on the primary culture of rat cortical neurons. Further studies on structure–activity relationship concerning substituted biphenyl molecules are under way.

Acknowledgements

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