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Synthesis of riccardin C and its seven analogues. Part 1: The role of their phenolic hydroxy groups as LXRα agonists

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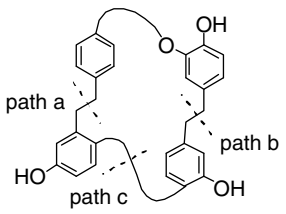
ABSTRACT

Riccardin C, a nuclear receptor LXRα selective agonist, is an 18-membered macrocyclic bisbibenzyl isolated from several liverworts. Synthesis of riccardin C and its seven *O*-methylated derivatives was accomplished. The synthetic sequence highlights an intramolecular Suzuki–Miyaura coupling in the formation of the 18-membered biaryl linkage present in riccardin C. The structure–activity relationship of these compounds suggests that all of the phenolic hydroxy groups present in riccardin C are essential for the activation of LXRα.

Macrocyclic bisbibenzyls such as marchantins, riccardins and plagiochins are phenolic constituents that are characteristic of liverworts and exhibit a variety of biological activities.[1] Riccardin C (**1**) was originally isolated from *Reboulia hemisphaerica* (L.) Raddi[2a] and was later found in several liverworts.[2] Recently riccardin C was discovered to bind directly to liver X receptor α (LXRα), a member of the nuclear receptor superfamily, leading to the activation of LXRα/RXR-dependent reporter gene transcription.[3] Interestingly, riccardin C functions as an antagonist but not an agonist of LXRβ. In addition, it has no ability to activate other nuclear receptors, such as PPARγ, RARα, RARβ, RARγ, FXR, and RXRα, and increases LXR-target gene expression in macrophages. From these results riccardin C was expected to be a lead compound for the treatment of cardiovascular-related diseases such as arteriosclerosis because LXRs are thought to regulate cholesterol metabolites.[4] In this paper, we report our independent synthesis of riccardin C and its seven *O*-methylated analogues and their selective manner of binding to LXRs, α and β, and discuss the role of three hydroxy groups related to the activation of LXRs through structure–activity relationship of the synthesized compounds.

Four groups have reported the synthesis of riccardin C (**1**). The key step in the synthesis of **1** is the construction of the 18-membered macrocyclic ring. All of their formations of the corresponding 18-membered ring were employed at the benzilic position (paths a and b in Fig. 1) by Wurtz,[5a] Wittig-type[5b,d], and McMurry reaction.[5c] Previously we reported the synthesis of plagiochin A and D,[6a,b] and isoplagiochin D,[6c] which are variant members of the 16-membered macrocyclic bisbibenzyls with a biaryl linkage, by applying intramolecular Stille–Kelly or Suzuki–Miyaura reaction as the key step to form the biaryl linkage. Our approach aiming



riccardin C (**1**)

Figure 1. Riccardin C (**1**), a LXRα receptor agonist. Paths a and b: macrocyclization points to synthesize **1** by other groups. Path c: a macrocyclization point in this study.

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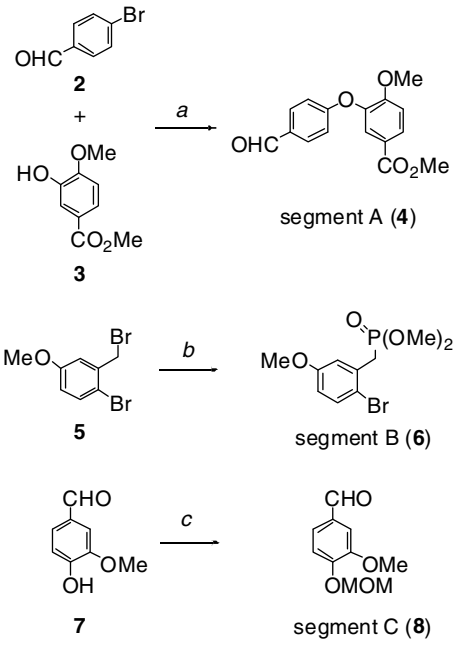


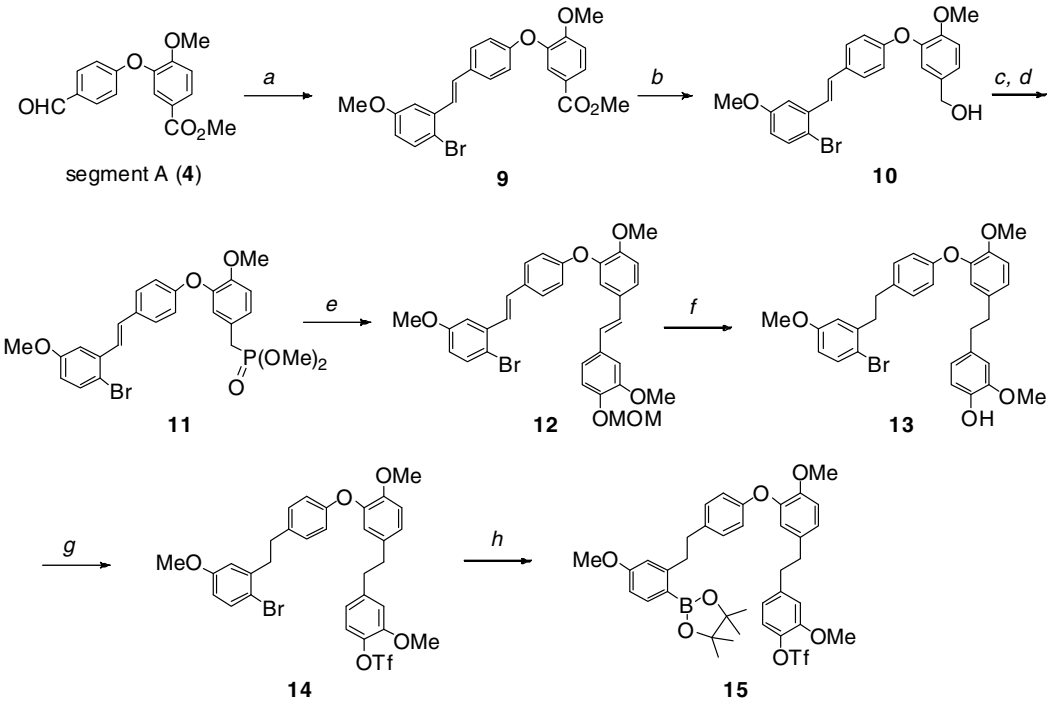
Scheme 1. Preparation of all building blocks **4**, **6** and **8** for the synthesis of riccardin C. Reagents and conditions: (a) K_2CO_3, DMSO, 140 °C, 4 h, 68%; (b) $P(OMe)_3$, 90 °C, 2 h, 98%; (c) MOMCl, NaH, THF, 1.5 h, 84%.

at synthesis of the biaryl-type macrocyclic bibenzyls is most likely to follow the biosynthetic pathway involved in the series of macrocyclic bisbibenzyls.[7] Thus we have applied the same strategy (path c in Fig. 1) for the synthesis of riccardin C as part of our studies on natural product synthesis utilizing transition metal catalysts.[8]

The synthesis commenced with preparation of segments A (**4**), B (**6**), and C (**8**) from commercially available chemicals in one step, respectively, according to the literature precedents shown in Scheme 1. Segment A was prepared by nucleophilic *ipso* substitution at the C-4 position of 4-bromobenzaldehyde (**2**) with methyl 3-hydroxy-4-methoxybenzoate (**3**).[9] Michaelis–Arbuzov reaction of 2-bromo-5-methoxybenzyl bromide (**5**) afforded segment (**6**)[6a] in good yield. Protection of the phenolic hydroxy group in vanilin (**7**) as a methoxymethyl (MOM) ether provided segment C (**8**).[10]

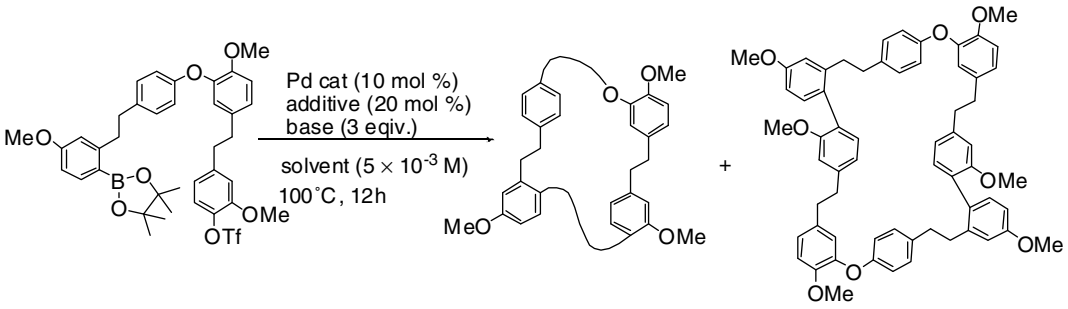
With all segments in hand, a precursor for macrocyclization was prepared by successive Horner–Wadsworth–Emmons (HWE) reactions shown in Scheme 2. Segment A was coupled with segment B by the first HWE reaction to give **9** in 98% yield. The ester group in **9** was reduced by $LiAlH_4$ and the resulting hydroxy group in **10** was converted to phosphonate **11** by consecutive bromination and phosphorylation. The second HWE reaction of **11** with segment **C** also proceeded smoothly to give **12** which have all carbon atoms required for the synthesis of riccardin C. Two double bonds in **12** have to be reduced with keeping a bromine atom on an aromatic group intact. After several unsuccessful attempts of the selective hydrogenation under H_2 atmosphere by using Pd or Pt catalyst, the olefins in **12** were selectively reduced by using triethylsilane in trifluoroacetic acid[11] at 60 °C. A MOM group was also removed during the reaction. The resulting phenolic hydroxy group in **13** was converted to trifluoromethanesulfonate **14** in 96% yield. Selective halogen-boronate exchange is required in order to prepare the cyclization precursor **15** even in the presence of aryltrifluoromethanesulfonyl group. The reaction selectively proceeded by treatment of **14** with bis(pinacolato)diboron and 10 mol % of $Pd(PPh_3)_4$ affording the desired product **15** in 95% yield.

The crucial macrocyclization Suzuki–Miyaura coupling to form the 18-membered macrocyclic ring was examined. The employed reaction conditions are shown in Table 1. $Pd(PPh_3)_4$ (10 mol %)–K_3PO_4–DMF system, which was effective for the synthesis of isoplagiochin D,[6c] was employed for this cyclization at first. The desired product **16**, however, was obtained in only



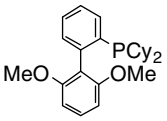
Scheme 2. Preparation of cyclization precursor **15**. Reagents and conditions: (a) NaH, THF,10 min, 0 °C then segment B (**6**), 20 h, 99%; (b) $LiAlH_4$, THF, 0.5 h, 90%; (c) NBS, Me_2S, CH_2Cl_2; 2 h –10 °C to rt; (d) $P(OMe)_3$, 100 °C, 1 h, 98% (2 steps); (e) NaH, THF, 10 min, 0 °C, then segment B, 3 h 87%; (f) Et_3SiH, TFA, 60 °C, 25 min, 74%; (g) *N*-phenylbis(trifluoromethanesulfonimide), Cs_2CO_3, MeCN/DMF (9/1), 8.5 h, 96%; (h) 10 mol % $Pd(PPh_3)_4$, bis(pinacolato)diboron, K_3PO_4, dioxane, 100 °C, 4.5 h, 95%.

Table 1
Macrocyclization by Suzuki–Miyaura coupling



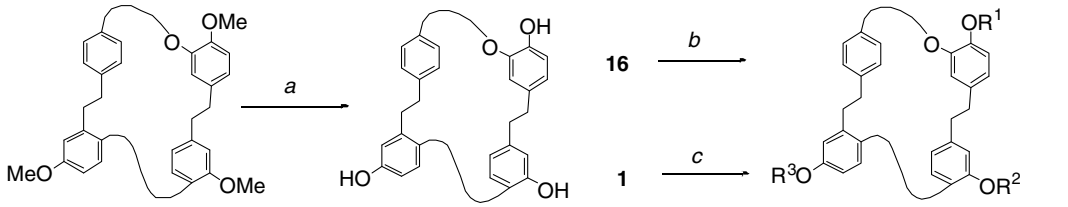
15 **16** **17**

Entry	Pd cat.	Additive	Base	Solvent	Isolated yield (%)	
					16	**17**
1	$Pd(PPh_3)_4$		K_3PO_4	DMF	9	
2	$Pd_2(dba)_3$	SPhos (**18**)[a]	K_3PO_4	DMF	16	
3	$Pd_2(dba)_3$	SPhos (**18**)[a]	Na_2CO_3[b]	DMF	37	27
4	$Pd_2(dba)_3$	SPhos (**18**)[a]	Na_2CO_3[b]	THF	34	24
5	$Pd_2(dba)_3$	SPhos (**18**)[a]	Na_2CO_3[b]	DMSO	3	
6[c]	$Pd_2(dba)_3$	SPhos (**18**)[a]	Na_2CO_3[b]	DMF	39	33
7[d]	$Pd_2(dba)_3$	SPhos (**18**)[a]	Na_2CO_3[b]	DMF	15	4

[a]


SPhos (**18**)

[b] 3 equiv of 2 M aqueous Na_2CO_3 solution were added.
[c] 20 mol % of $Pd_2(dba)_3$ and 40 mol % of SPhos were used.
[d] Higher diluted conditions were applied (2.5×10^{-3} M) and the reaction period was 24 h.



16 **1** **19a - 19f**; R^1, R^2, R^3 = H or Me
6 possible analogues

Scheme 3. Synthesis of riccardin C (**1**) and its six analogues. Reagents and conditions: (a) 12 equiv of BBr_3, CH_2Cl_2, 0 °C to rt, 7 h, 97%; (b) 5 equiv of BBr_3, CH_2Cl_2, 0 °C to rt, 20 min, yields are shown in Table 2; (c) 12 equiv of trimethylsilyldiazomethane, Et_2O, 20 h, yields are shown in Table 2.

9% yield (entry 1 in Table 1). The yield was improved when SPhos (**18**), a highly effective phosphine ligand for the preparation of extremely hindered biaryls in Suzuki–Miyaura coupling developed by Buchwald et al.,[12] was applied to our reaction (entry 2). This macrocyclization was highly dependent on the base. Na_2CO_3 enhanced reactivity to give desired product **16** in 37%

Table 2
Isolated yields of **19a–f**

Compound	R^1	R^2	R^3	Isolated yield (%)	
				From **16**	From **1**
19a (riccardin A)	H	H	Me	14	8
19b	H	Me	H	21	7
19c (riccardin F)	Me	H	H	—	10
19d	H	Me	Me	22	—
19e	Me	H	Me	—	10
19f	Me	Me	H	—	5

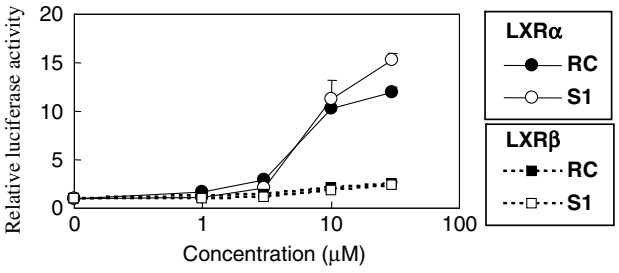


Figure 2. Activation of LXRα and LXRβ by natural and synthetic riccardin C. COS-1 cells were transfected with a reporter plasmid (pLXREX4-tk-Luc) and expression plasmids for LXRα (or LXRβ) and RXRα together with a plasmid for β-galactosidase as an internal control, and were treated for 24 h with various concentrations of natural riccardin C (**RC**) or synthetic riccardin C (**S1**) in the presence of compactin which depletes endogenous LXR ligands. The luciferase activity in the cell extracts was normalized using β-galactosidase and expressed as the fold induction relative to the vehicle-treated cells.

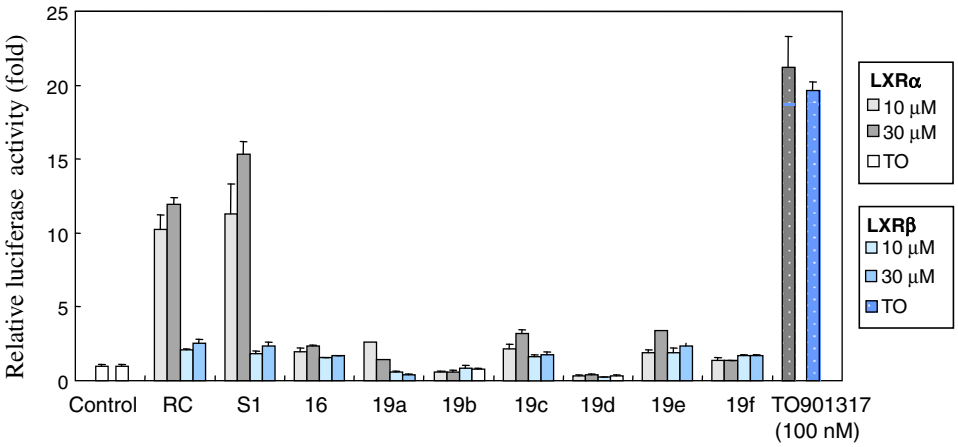


Figure 3. Agonistic activities on LXRα and LXRβ by natural (**RC**) and synthetic riccardin C (**S1**) and seven analogues (**16** and **19a–f**). TO901317 was used as a positive control. Experimental procedure is same as that described in the captions for Figure 1.

yield along with 27% of cyclic dimer **17** (entry 3). The yield was reduced in THF or DMSO (entries 4 and 5). Larger amounts of Pd catalyst (20 mol %) and SPhos (40 mol %) gave slightly improved yields (entry 6). Higher diluted conditions were not effective for suppressing the dimerization (entry 7).

Finally, treatment of **16** with BBr_3 removed three *O*-methyl groups giving rise to riccardin C in 97% yield (Scheme 3). The spectroscopic data of synthetic compound **1** were in agreement with those of the natural riccardin C.

In our previous study, riccardin F (**19c**), a 14-methoxy derivative of riccardin C, activated neither LXRα nor LXRβ.[3] This drastic difference in the activity between riccardin C and F prompted us to synthesize partially *O*-methylated analogues to explore the structure–activity relationship of three hydroxy groups. Treatment of permethylated riccardin C (**16**) with 5 equiv of BBr_3 led to three partially *O*-methylated compounds **19a**, **19b**, and **19d**. On the other hand, reaction of riccardin C with 12 equiv of trimethylsilyldiazomethane provided five compounds **19a**, **19b**, **19c, 19e**, and **19f**. The isolated yields of these analogues are shown in Table 2. Their structures were carefully determined by NOESY spectroscopic analyses and comparison with the spectroscopic data of natural riccardins C and F. Preparation of all possible partially *O*-methylated analogues were completed by these reactions.

We first confirmed the LXRs agonistic activity of synthetic riccardin C by comparing it with that of natural riccardin C. Upon cotransfection with LXRα/RXR, 30 mmol L^{-1} of synthetic riccardin C raised the transactivation of an LXR-response element-driven reporter gene by approximately 15-fold. The dose–response curve was in good accordance with that of natural riccardin C shown in Figure 2. In addition, neither of them activated LXRβ. Synthetic analogues of riccardin C were also screened by the transient transfection assay shown in Figure 3. Unexpectedly, none of them activated LXRα or LXRβ. These results clearly indicate that all hydroxy groups in riccardin C are essential for its binding to LXRα.[13]

In conclusion, riccardin C and seven analogues were synthesized, via the intramolecular Suzuki–Miyaura coupling to construct the necessary 18-membered biaryl linkage. Three phenolic hydroxy groups in riccardin C were clarified to be indispensable for binding to LXRα according to structure–activity relationship studies. The present structure–activity relationship studies on riccardin C provide beneficial information for the design and synthesis of more potent and selective LXRα agonists.

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References and notes

1. Asakawa, Y.. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer: Vienna, 1995; Vol. 65, pp 618–621.
2. (a) Asakawa, Y.; Matsuda, R. *Phytochemistry* **1982**, *21*, 2143; (b) Lu, Z. Q.; Fan, P. H.; Ji, M.; Lou, H. X. *J. Asian Nat. Prod. Res.* **2006**, *8*, 187; (c) Harinantenaina, L.; Asakawa, Y. *Chem. Pharm. Bull.* **2004**, *52*, 1382; (d) Bardón, A.; Kamiya, N.; Toyota, M.; Takaoka, S.; Asakawa, Y. *Phytochemistry* **1999**, *52*, 1323; (e) Anton, H.; Schoeneborn, R.; Mues, R. *Phytochemistry* **1999**, *52*, 1639. and references cited therein.
3. Tamehiro, N.; Sato, Y.; Suzuki, T.; Hashimoto, T.; Asakawa, Y.; Yokoyama, S.; Kawanishi, T.; Ohno, Y.; Inoue, K.; Nagao, T.; Nishimaki-Mogami, T. *FEBS Lett.* **2005**, *579*, 5299.
4. Li, A. C.; Glass, C. K. *J. Lipid Res.* **2004**, *45*, 2161.
5. (a) Gottsegen, Á.; Nógrádi, M.; Vermes, B.; Kajtár-peredy, M.; Bihátsi-karsai, É. *Tetrahedron Lett.* **1988**, *29*, 5039; (b) Eicher, T.; Fey, S.; Puhl, W.; Büchel, E.; Speicher, A. *Eur. J. Org. Chem.* **1998**, *1998*, 877; (c) Harrowven, D. C.; Woodcock, T.; Howes, P. D. *Angew. Chem., Int. Ed.* **2005**, *44*, 3899; (d) Dodo, K.; Aoyama, A.; Noguchi-Yachide, T.; Makishima, M.; Miyachi, H.; Hashimoto, Y. *Bioorg. Med. Chem.* **2008**, *16*, 4272.
6. (a) Fukuyama, Y.; Yaso, H.; Nakamura, K.; Kodama, M. *Tetrahedron Lett.* **1999**, *40*, 105; (b) Fukuyama, Y.; Yaso, H.; Mori, T.; Takahashi, H.; Minami, H.; Kodama, M. *Heterocycles* **2001**, *54*, 259; (c) Esumi, T.; Wada, M.; Mizushima, E.; Sato, N.; Kodama, M.; Asakawa, Y.; Fukuyama, Y. *Tetrahedron Lett.* **2004**, *45*, 6941.
7. (a) Friederich, S.; Maier, U. H.; Deus-Neumann, B.; Yoshinori Asakawa, H.; Zenk, M. *Phytochemistry* **1999**, *50*, 589; (b) Keserü, G. M.; Nógrádi, M. *Phytochemistry* **1992**, *31*, 1573.
8. Esumi, T.; Zhao, M.; Kawakami, T.; Fukumoto, M.; Toyota, M.; Fukuyama, Y. *Tetrahedron Lett.* **2008**, *49*, 2692. and references cited therein.
9. Ha, T. T. N.; Nogradi, M.; Brlik, J.; Kajtar-Peredy, M.; Wolfner, A. *J. Chem. Res. Synop.* **1991**, 137.
10. Mabic, S.; Vaysse, L.; Benezra, C.; Lepoittevin, J. *Synthesis* **1999**, 1127.
11. Kursanov, D. N.; Parnes, Z. N.; Bassova, G. I.; Loim, N. M.; Zdanovich, V. I. *Tetrahedron* **1967**, *23*, 2235.
12. (a) Walker, S. D.; Barder, T. E.; Martinelli, J. R.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2004**, *43*, 1871; (b) Barder, T. E.; Walker, S. D.; Martinelli, J. R.; Buchwald, S. L. *J. Am. Chem. Soc.* **2005**, *127*, 4685.
13. Recently, Hashimoto et al. reported that riccardin C had no agonistic activity toward LXRs by using a chimeric receptor system in which the ligand binding domain of LXR was fused to the yeast GAL4 DNA binding domain (Ref. 5d). In contrast, in the present and previous studies, we have shown the agonist activity of riccardin C in a physiological assay system using a heterodimer of full-length LXR and RXR and an LXR-responsive element-driven reporter gene.