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Syntheses of 5′-*O*-desmethylterphenyllin and related *p*-terphenyls and their inhibitory activity of TNF-α release from RBL-2H3 cells

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ABSTRACT

The first total syntheses of 5′-*O*-desmethylterphenyllin and three related *p*-terphenyls have been achieved. The methodology features a Suzuki–Miyaura coupling reaction with a hindered aryl chloride as the key step. Two of the four synthesized 5′-*O*-desmethylterphenyllins exhibit moderate TNF-α release-inhibitory activity toward RBL-2H3 cells.

A number of bioactive polyhydroxy-*p*-terphenyls have been isolated from various microorganisms.[1] There are several groups of bioactive, naturally occurring *p*-terphenyls with common structure features. One group of polyhydroxy-*p*-terphenyls is featured by an acylated catechol structure in the center ring such as terrestrins,[2] vialinin A,[3] and thelephantins (Fig. 1). Although vialinin A (**1**) was originally isolated from the edible mushroom *Thelephora vialis* as a DPPH radical inhibitor,[3] it was also shown to be a potent inhibitor of tumor necrosis factor (TNF)-α release from rat basophilic leukemia (RBL)-2H3 cells.[4] Thelephantin O (**2**) is an acylated *p*-terphenyl isolated from *Thelephora aurantiotincta*, and that is cytotoxic against HepG2 and Caco2.[5] The second group of polyhydroxy-*p*-terphenyls consists of prenylated *p*-terphenyls (Fig. 1) and includes terprenins[6] (**3**) and prenylterphenyllins[7] (**4**) isolated from *Aspergillus candidus* RF-5672 and *A. candidus* IF10, respectively. Terprenin was reported to be a strong immunosuppressive agent,[6] and the phenylterphenyllins were reported to be cytotoxic.[7]

In addition, other *p*-terphenyls without acyl or prenyl groups have been isolated from some fungi. In 1975, Marchelli and Vining isolated terphenyllin (**5**) from *A. candidus*,[8] and **5** was subsequently shown to have anticancer,[9] DPPH radical scavenging,[10] and HIV-1-integrase inhibitory[11] activities. Recently, Lin and She co-worker isolated 5′-*O*-desmethylterphenyllin (**6**), together with its hydroxylated derivatives **7** and **8**, from the mangrove endophytic fungus *Penicillium chermesinum* (ZH4-E2) and found these compounds to be α-glucosidase inhibitors.[12] Meanwhile, Ge and co-workers also reported the isolation of 5′-*O*-desmethylterphenyllin (**6**) from endophytic *Aspergillus* sp. YXf3.[13] Compound **7** was also isolated from the sclerotia of *P. raistrickii* and was reported to reduce the growth rate of the larvae of the corn earworm *Helicoverpa zea*.[14] In addition, Takahashi and Abe et al. reported that DMT (**10**), a simplified synthetic analogue of vialinin A, exhibited potent inhibitory activity—as high as that of vialinin A—against TNF-α release from RBL-2H3 cells.[15] Although no

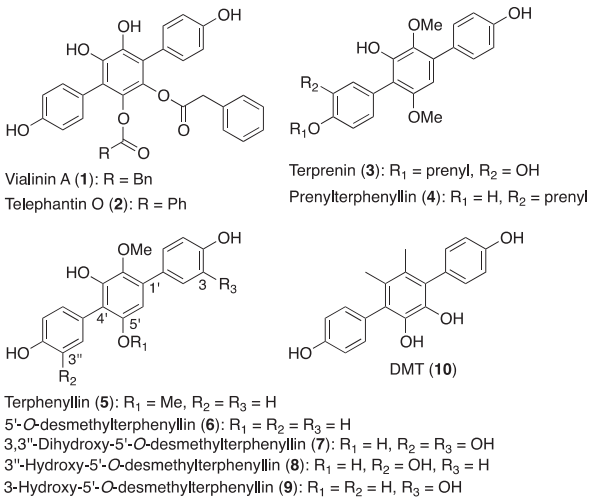

Figure 1. Structures of *p*-terphenyls.

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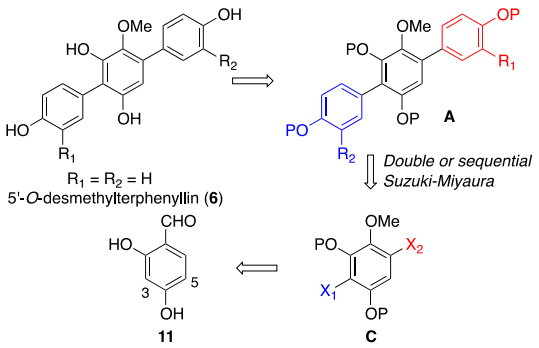


Scheme 1. Retrosynthetic analysis of 5′-*O*-desmethylterphenyllins.

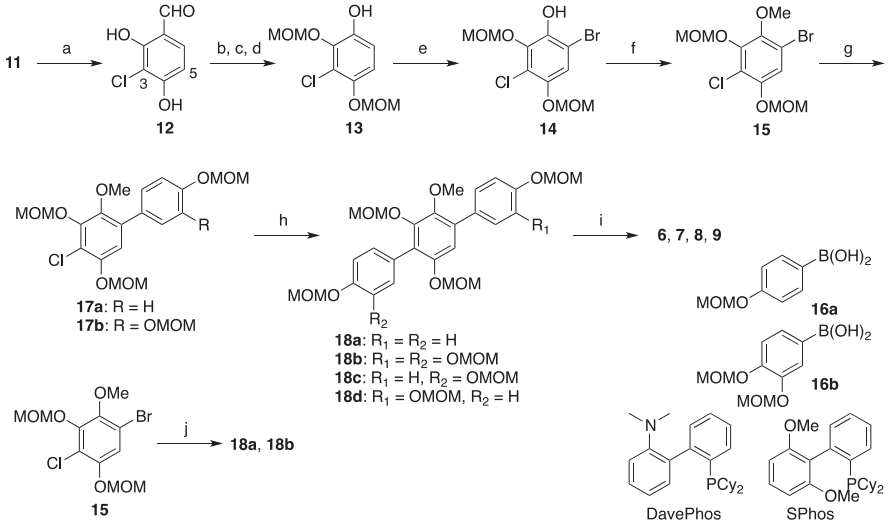
immunosuppressive activity for 5′-*O*-desmethylterphenyllin and its related *p*-terphenyls has yet been reported, we proposed that these compounds should have immunosuppressive or inhibitory activity against TNF-α release because of their structural similarity to DMT, terphenyllins, and terprenins.

Recent advances in techniques used in chemical biology prompted synthetic organic chemists to elucidate the mode of action and target molecules of bioactive natural products.[16] Very recently, Yajima and co-workers identified the binding protein for vialinin A and DMT, ubiquitin-specific peptidase 5 (USP5), using a biotinylated derivative of DMT.[17] Using chemical probes derived from DMT, it should be possible to fully elucidate the unique pathway of this immunosuppressive effect, which is different from that of FK-506. However, because DMT contains a catechol moiety in its central aromatic ring, it is relatively sensitive to oxidation under aerobic conditions, which is disadvantageous in the preparation of chemical probes. However, because **6** is not a catechol derivative, it would be expected to be more stable than DMT against oxidation. Thus, we propose that **6** is a promising alternative for DMT both as a template for chemical probes for the identification of the target protein and as a candidate for the development of immunosuppressive agents. Therefore, to confirm the immunosuppressive activity of 5′-*O*-desmethylterphenyllins, we synthesized **6**, **7**, **8**, and the unnatural derivative **9** by employing Suzuki–Miyaura coupling reactions as the key steps.

The retrosynthetic analysis for **6** is based on double or sequential Suzuki–Miyaura cross-couplings, as shown in Scheme 1. Considering the vast number of available methods for the introduction of various aryl groups, the incorporation of different functional groups required for the Suzuki–Miyaura coupling reactions in the center ring is favorable. For example, in the total synthesis of terprenin by Kawada and Ohtani, bromo and iodo groups were installed in the central aryl core, and sequential Suzuki–Miyaura cross couplings followed.[18]

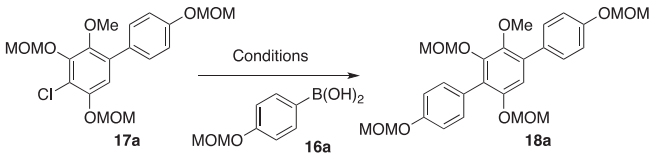
The synthesis began with commercially available **11** (Scheme 2). However, a preliminary study showed that it was difficult to introduce the bromo or iodo group at the C-3 position of **11** under standard conditions, such as with bromine or iodine and NBS or NIS. Although there have been some reports on the synthesis of 3-bromo derivatives of **11** employing sodium hypobromite as the bromine source,[19] we obtained only the 5-bromo derivative as a major product. Fortunately, using Liebert's method,[19] the regioselective chlorination of **11** using sodium hypochlorite produced **12** in high yield. Although the Suzuki–Miyaura coupling with a sterically hindered aryl chloride was anticipated to be relatively difficult, we decided to employ the aryl chloride as the key intermediate in the total synthesis and take on the challenge of developing efficient conditions for the coupling reaction that would be appropriate for natural product synthesis. Therefore, the hydroxy groups of **12** were protected as MOM ethers, and subsequent Baeyer–Villiger oxidation and methanolysis produced **13**, as shown in Scheme 2. The halogenation of the *ortho* position of the phenol was again troublesome. However, we found that pyridinium hydrobromide perbromide was effective, producing the corresponding bromide in 64% yield. The methylation of the hydroxy group of **14** afforded the key intermediate **15** (X_1 = Cl, X_2 = Br in **C**). With the key intermediate **15** in hand, the regioselective Suzuki–Miyaura coupling at the bromo moiety with boronic acid **16a** was investigated. As expected, no reaction was observed at the chloro substituent under all standard conditions, and the corresponding biphenyl **17a** was chemoselectively obtained in good yield by employing a catalyst generated from $Pd(OAc)_2$ and DavePhos.[20]

Next, the second Suzuki–Miyaura coupling of the sterically hindered chloro moiety of **17a** was investigated. Although there have been various reports on the Suzuki–Miyaura reaction of aryl chlorides employing palladium catalysts with a wide variety of



Scheme 2. Synthesis of 5′-*O*-desmethylterphenyllins. Reagents, conditions, and yields: (a) NaClO aq, NaOH (Ref. 19), 95%; (b) MOMCl, DIPEA, DMF, 99%; (c) *m*-CPBA, CH_2Cl_2, reflux; (d) Et_3N, MeOH, 97% in two steps; (e) PyHBr-Br_2, py, 0 °C to rt 63%; (f) MeI, K_2CO_3, acetone, 45 °C, 99%; (g) $Pd(OAc)_2$, DavePhos, K_3PO_4, toluene, 90 °C, **16a** for **17a**, 83% or **16b** for **17b**, 71%; (h) $Pd(OAc)_2$, SPhos, NaOH, toluene, 95 °C, **16a** for **18a**, 95%, or **16b** for **18b**, 83%, **16a** for **18c**, 93%, or **16b** for **18d**, 84%; (i) HCl aq, MeOH, 83% for **6** or HCl, MeOH, 71% for **7**, 86% for **8**, or 98% for **9**; (j) $Pd(OAc)_2$, SPhos, NaOH, toluene, 95 °C, **16a** for **18a**, 92%, or **16b** for **18b**, 89%.

Table 1
Suzuki–Miyaura couplings generating *p*-terphenyl **18a**[a]



Entry	Catalyst/ligand	Base	Yield (%)
1	$Pd(OAc)_2/P(t\text{-}Bu)_3$	NaOH	—[b]
2	$Pd(OAc)_2$/XPhos	NaOH	14
3	$Pd(OAc)_2$/DavePhos	NaOH	24
4	$Pd(OAc)_2$/SPhos	NaOH	95
5	$Pd(OAc)_2$/SPhos	K_2CO_3	13
6	Pd-PEPPSI-IPent[c]	*t*BuOK	—[b]
7	$PdCl_2[P(t\text{Bu})_2C_6H_4NMe_2]_2$[d]	K_2CO_3	—[b]

[a] Conditions: 10 mol % palladium catalyst and 10 equiv base in toluene at 95 °C.
[b] Not observed.
[c] Reaction was performed at 65 °C.
[d] Reaction was performed at 100 °C in dioxane–water.

phosphine ligands,[21] the reaction with **17a** did not proceed well under the reported conditions (Table 1). Fortunately, we found the combination of $Pd(OAc)_2$ and the SPhos ligand[22] to be highly effective in this reaction during our screen of phosphine ligands. The desired terphenyl **18a** was obtained in 95% isolated yield under the optimized conditions (entry 4). Finally, the deprotection of the four MOM groups of **18a** with aqueous HCl afforded 5′-*O*-desmethylterphenyllin (**6**) (Scheme 2). The ^1H and ^{13}C NMR spectra were in good agreement with the reported data.[12,13] In the same manner, sequential Suzuki–Miyaura coupling reactions with boronic acids **16a** and **16b** produced the other three terphenyls, **18b**, **18c**, and **18d**. The deprotection of the MOM groups of these intermediates with methanolic HCl produced the two natural terphenyls 3,3″-dihydroxy-5′-*O*-desmethylterphenyllin[14] (**7**) and 3″-hydroxy-5′-*O*-desmethylterphenyllin[13] (**8**), as well as the unnatural terphenyl 3-hydroxy-5′-*O*-desmethylterphenyllin (**9**). The terphenyls **18a** and **18b** were also obtained via the double Suzuki–Miyaura coupling of **15** with **16a** and **16b**, respectively, using the palladium catalyst with SPhos. These results suggest that the established reaction condition would be one of the most powerful and useful conditions for Suzuki–Miyaura coupling.

Then, the TNF-α and β-hexosaminidase release-inhibitory activities of the four synthesized 5′-*O*-desmethylterphenyllins were evaluated for RBL-2H3 cells according to the previously reported procedure.[4] Figure 2 shows the results of the assays. As expected, compounds **6** and **7** were active against TNF-α release from RBL-2H3 cells, and the release of the granular enzyme β-hexosaminidase from RBL-2H3 cells[23] was weakly inhibited by **6**–**9** (IC_{50}: 1–10 μM). Although the IC_{50} values of **6** and **7** for anti-TNF-α release were comparable to vialinin A and DMT (0.09 and 0.02 nM respectively), the levels of activity reached saturation at approximately 1 nM. This phenomenon was also observed with the 2′,3′-bis-MOM ether derivative of DMT.[15] In contrast, vialinin A and DMT dose-dependently inhibited TNF-α release.[4,15] As described above, Yajima and co-workers identified USP5 as the target protein for vialinins, and vialinins strongly inhibited USP5 activity.[17] However, the above results suggest the possibility that the vialinins might inhibit additional proteins related to TNF-α release from RBL2H3 cells, while 5′-*O*-desmethylterphenyllins might inhibit only a single protein, such as USP5, thereby causing the saturation of the inhibitory activity. This hypothesis may be confirmed using 5′-*O*-desmethylterphenyllins as the backbones of molecular probes for the identification of their target molecules.

In summary, we achieved the first total syntheses of 5′-*O*-desmethylterphenyllin and three related *p*-terphenyls via Suzuki–Miyaura coupling reactions, and we confirmed that a palladium catalyst with SPhos ligand is highly effective in the Suzuki–Miyaura coupling of a hindered aryl chloride. In addition, we discovered new biological activity of 5′-*O*-desmethylterphenyllins, and the different behavior in their TNF-α release-inhibitory activity compared to that of the vialinins suggests that the target molecules for 5′-*O*-desmethylterphenyllins and vialinins might be different. Because our synthetic methodology is versatile and it is easy to install various aryl moieties into the central aryl group, this route should enable the development of more advanced immunosuppressive agents based on the 5′-*O*-desmethylterphenyllin core structure.

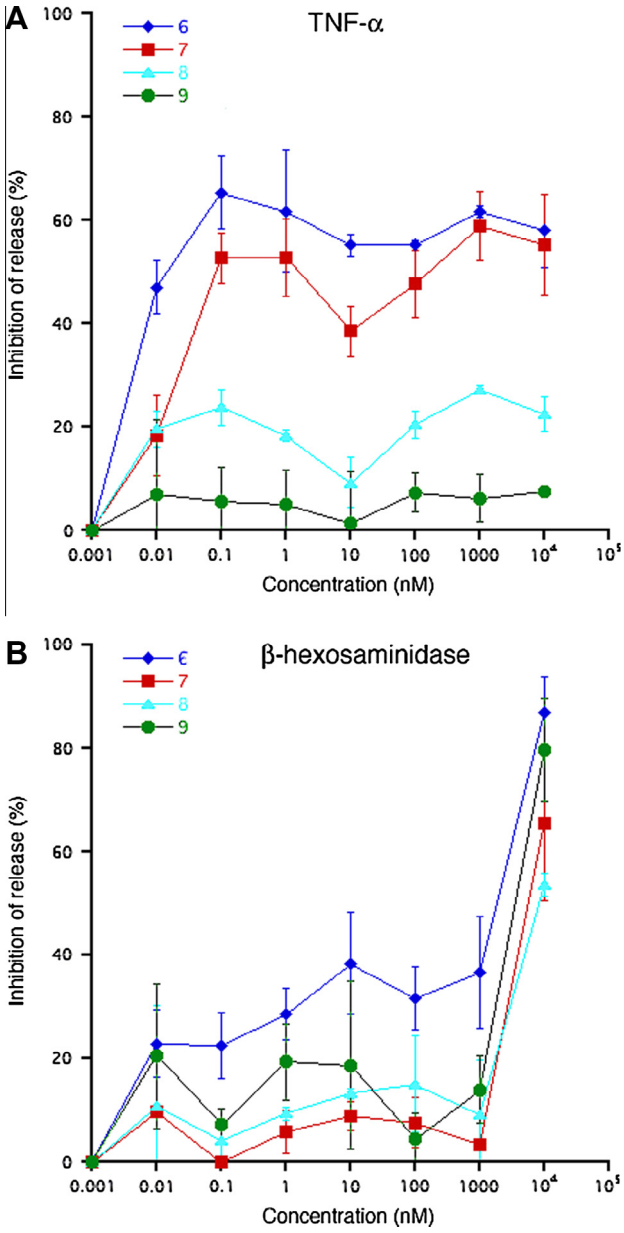


Figure 2. Effects of 5′-*O*-desmethylterphenyllins on TNF-α release (**A**) and β-hexosaminidase release (**B**) from RBL-2H3 cells. Each value represents the mean ± standard deviation of triplicate determinations.

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Supplementary data

Supplementary data (experimental procedures and ^{1}H and ^{13}C NMR spectra of the synthetic compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.07.062.

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