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Synthesis of polyozellin, a prolyl oligopeptidase inhibitor, and its structural revision

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ABSTRACT

Polyozellin is a *p*-terphenyl compound which was isolated from *Polyozellus multiplex*, and exhibits an inhibitory activity against prolyl oligopeptidase (POP). Its structure was assigned as **1** having a *p*-terphenyl skeleton including a *p*-substituted dibenzofuran moiety by spectroscopic analyses and chemical means. This paper describes the total syntheses of the proposed structure **1** for polyozellin and its *o*-isomer **2**, revising the structure of polyozellin to the latter. These syntheses involved a double Suzuki-Miyaura coupling using chlorophenylboronic acid as a common key building block, and Cu mediated Ullmann cyclization as key steps. The inhibitory activities of synthetic compounds against POP and cancer cells were also evaluated.

Prolyl oligopeptidase (POP: EC3.4.21.26) is a serine protease that hydrolyses bioactive peptides of less than 30 amino acids such as substance P and vasopressin at the carboxyl side of proline resisue.[1,2] Recent studies have revealed that the enzyme participates in several functions of the central nervous system and that it is a target in memory and neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (PD).[3,4] PD is a neurodegenerative disorder and may be related to the aggregation of α-synuclein (α-syn: 140 amino acids), a brain protein with functions in neurotransmitter packing and release.[5] Lewy bodies, histopathological hallmarks of PD, consist mostly of aggregated α-syn and they are the toxic species.[6] POP can increase the aggregation of α-syn via direct protein-protein interaction and the inhibitor reduces its aggregation *in vitro* and *in vivo*.[7,8] Additionally, the POP inhibitor has effects on autophagy induction and decrease in α-syn dimerization.[9] It has also been reported that POP inhibition suppresses the growth of human neuroblastoma cell line, NB-1 and human gastric cancer cell line, KATO III, respectively.[10,11] Therefore, POP inhibitors are a promising tool for the prevention and treatment of neurodegenerative diseases and cancer. Such consideration has stimulated researchers to search for new drug candidates from natural sources.[12] In 1997, Yoo et al. isolated a POP inhibitor from the Korean mushroom *Polyozellus multiplex*, and named it polyozellin.[13] The structure was mainly determined by NMR and derivatization studies to be **1** possessing a unique *p*-terphenyl dibenzofuran structure (Fig. 1). The natural product inhibited POP dose-dependently (IC_{50} = 2.72 μM), and its effect on the enzyme activity was shown to be a noncompetitive inhibition. Since then, it has been reported that polyozellin exhibits a variety of interesting biological activities such as induction of Phase 2 enzymes,[14] inhibition of NO (nitric oxide) production,[15] anti-inflammatory effects,[16] antiseptic effects,[17] increasing the glutamate-treated HT22 cell viability,[18] and inhibition of TACE (tumor necrosis factor-α-converting enzyme).[19] Recently, we have been engaged in synthetic studies on bioactive *p*-terphenyls, resulting in the total synthesis of vialinins A,[20] B,[21] and kynapcin-12.[22] As part of our continuing studies in this field, we describe herein the total synthesis of polyozellin which dictates the revision of the formula to **2**.

Our synthetic strategy[23] directed towards **1** included a double Suzuki-Miyaura coupling of *p*-benzoquinone **3** with chlorophenylboronic acid **4**,[21] and an intramolecular double Ullmann reaction of *p*-hydroquinone **6b** as key steps (Scheme 1). Although the Suzuki-Miyaura coupling[24] and Ullmann reaction[25] are powerful tools for the preparation of oligoarenes, few paper has appeared dealing with synthesis of highly oxygenated benzo[1,2-*b*:4,5-*b*′]bisbenzofurans by utilizing such reactions.[26] Therefore this strategy would be challenging, and of interest from the standpoint of organic synthesis. The starting quinone **3** was prepared[27] from a commercially

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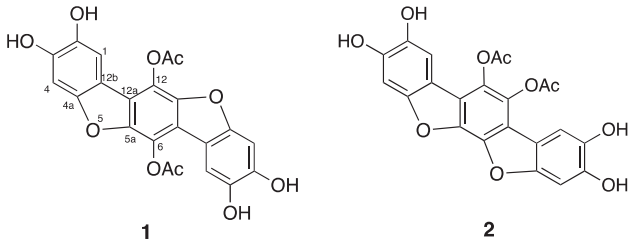
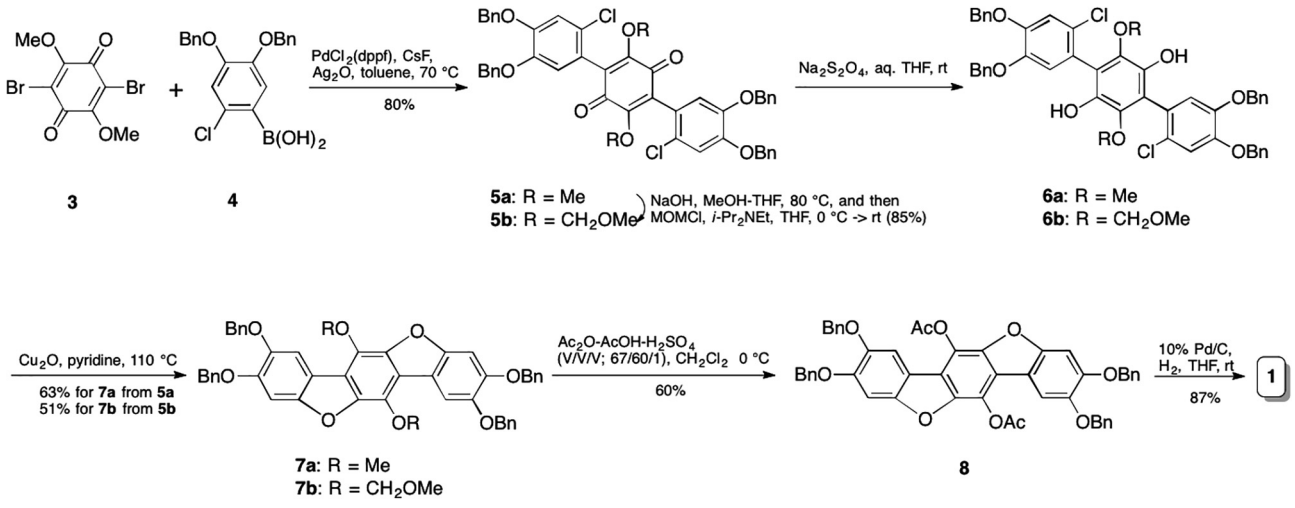


Fig. 1. The proposed structure **1** for polyozellin and its revised form **2**.

available 2,5-dimethoxy-*p*-benzoquinone. The Suzuki-Miyaura coupling of **3** with **4** was accomplished by using $PdCl_2$(dppf) in the presence of CsF and Ag_2O in toluene to afford *p*-terphenyl **5a**[28] in high yield. In this coupling, the addition of Ag_2O was crucial to prevent debromination of **3**.[29] The quinone moiety in **5a** was reduced with sodium dithionite to give hydroquinone **6a**, which was immediately employed in the next cyclization without purification. The reaction of **6a** with $Pd(OAc)_2$-XPhos[30] in the presence of potassium acetate in toluene-DME caused an oxidation, resulting in the starting quinone **5a**. A CuO[31] mediated cyclization in pyridine provided **7a** in 10–30% yield. The use of Cu_2O[32] improved the reaction to afford the desired compound in good yield, demonstrating the usefulness of this catalyst for the double Ullmann reaction. The structure of **7a** was confirmed by 2D-NMR spectra.[33] Contrary to our expectations, all attempts for de-methylation of **7a** failed. For example, treatment of **7a** with BCl_3 followed by acetylation afforded a monoacetate with one remaining methoxy group. The reaction of a thiolate anion such as *n*-PrSLi and EtSNa led to a complex mixture. Therefore, we next exchanged the *O*-protecting group from Me to MOM. The replacement was initiated by hydrolysis of **5a** under alkaline conditions,[34] and the resulting diosphenol was immediately submitted to methoxymethylation to give **5b**[35] in high yield. According to the method described above, this compound was transformed into the corresponding MOM analog **7b**[33] in 2 steps. De-methxoymethylation of **7b** with HCl/MeOH or $BF_3{\cdot}Et_2O$-methylsulfide, gave diacetate **8** after acetylation but the yield was very low (~10%). This result might reflect the instability of the intermediary *p*-hydroquinone[36] and its low reactivity toward acetylation. In order to avoid the isolation of the unstable *p*-hydroquinone, direct conversion of **7b** into **8** was examined. Acetolysis[37] of **7b** yielded a promising result and the desired acetate **8** was obtained in a practical yield. Finally, **8** was hydrogenated over 10% Pd/C in THF to give **1**. The ^{1}H NMR spectral data of the synthetic sample were inconsistent with those of natural polyozellin (Table 1). There were also some discrepancies in the ^{13}C NMR spectra data; natural polyozellin exhibited three sp^2 carbons C-5a, C-12 and C-12a of the central aromatic ring at δ 137.59, 130.80, and 116.94 whereas those of synthetic **1** were observed at 143.54, 125.03 and 117.27 ppm, respectively. These data suggest the structural difference of the central ring, and that the proposed structure of natural polyozellin should be revised. In nature, *o*-terphenyl derivatives have not been found, and very few *m*-terphenyls occur. Taking this finding together with the NMR data reported, we speculated that natural polyozellin would have a symmetrical *p*-terphenyl structure, and an *o*-isomer **2** of the original form was proposed as the real structure. The synthesis began from a key intermediate **9**[20] in our total synthesis of vialinin A (Scheme 2). A double coupling with **4** was effected by using Pd $(Ph_3P)_4$ as a catalyst in the presence of Cs_2CO_3 in toluene to provide *p*-terphenyl **10**[28,38] in high yield. After treatment with HCl, the resulting catechol was heated with Cu_2O in pyridine. As expected, this cyclization also proceeded nicely to give *p*-dibenzofuran **11**[33] in good yield. Oxidation of the methylene acetal moiety with Pb $(OAc)_4$ followed by deacetylation of the resulting orthoacetate with an acid or a base was concomitant with oxidation of the catechol moiety,[36] resulting in the corresponding *o*-quinone as a major product. Although we obtained the desired diacetate **12** from the *o*-quinone *via* sodium dithionite reduction and acetylation, the overall yield was found to be quite low. Therefore, direct preparation of **12** from the orthoacetate was investigated. Its acetolysis gave a complex mixture, while acetylation under basic conditions provided **12**. All benzyl groups in **12** were removed by hydrogenolysis to afford **2** in good yield. The ^{1}H and ^{13}C NMR data of **2** were identical to those of natural polyozellin (Table 1), establishing the structure of the natural product as **2**.[39]

The inhibitory activities of **1** and **2** against POP and cancer cell lines (HL60 and MCF-7) were evaluated,[43] and the results are shown in Table 2. Both compounds exhibited a strong inhibitory activity against POP, which was comparable with positive control of propeptin. The inhibition potential of both compounds toward the growth of the tumor cells was shown to be moderate. Interestingly, there was no difference in the inhibitory activities between **1** and **2**. The planarity of the *p*-terphenyl skeleton rather than the position of the acyl function might be crucial for biological activity. These results might provide useful information for designing a new POP inhibitor as well as antitumor drugs.



Scheme 1. Synthesis of the proposed structure **1** for polyozellin.