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Concise synthesis of polymethoxyflavone sudachitin and its derivatives, and biological evaluations

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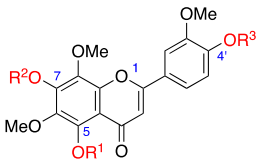
ABSTRACT

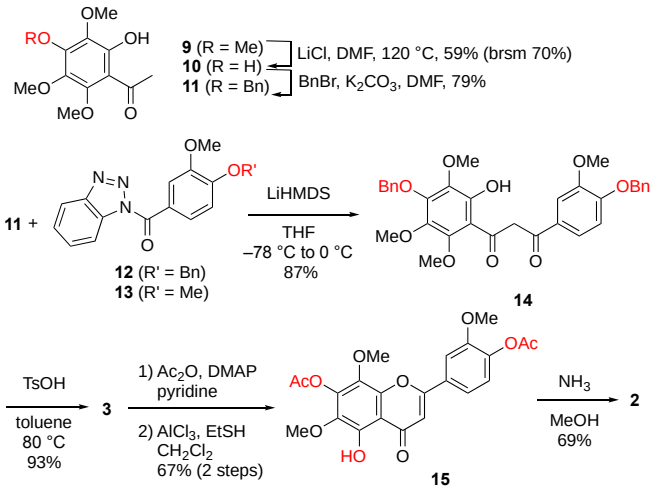
We accomplished a divergent synthesis of sudachitin (**2**), a polymethoxyflavone isolated from citrus fruits, and six derivatives from acetophenone **9**, which was an intermediate in our previous synthesis of nobiletin (**1**). Compound **2** enhanced glucose-induced insulin secretion in INS-1D cells, but was less potent than **1**. Compared with **1**, compound **2** and two derivatives were more potent inhibitors of cAMP-specific phosphodiesterase.

Polymethylated flavones from citrus fruits[1] have a variety of interesting physiological actions, including anti-inflammatory, anti-cancer, anti-apoptosis, anti-dementia and neuroprotective activities. Therefore, practical and flexible synthetic methods are needed to obtain sufficient amounts of derivatives for testing. During the course of our investigation of flavone synthesis,[2] we accomplished a practical and economical synthesis of nobiletin (**1**) on a hundred-gram scale.[3, 4] Here, we turned our attention to sudachitin (**2**), which is a 5,7,4'-tridesmethyl nobiletin derivative.[5-7] Although synthesis and biological evaluation of desmethyl derivatives on the A-ring of nobiletin have been reported by Oshitari and Natsugari's group, [8] there has been no detailed comparison of the biological activities of **1** and **2**. Furthermore, in order to elucidate the biosynthetic pathway of **2** from **1**, we planned to synthesize the possible biogenesis intermediates **3**–**8** using our established methodology.[2]



	R^1	R^2	R^3
Nobiletin (**1**)	Me	Me	Me
Sudachitin (**2**)	H	H	H
3	Me	H	H
4	Me	Me	H
5	H	Me	H
6	H	Me	Me
7	Me	H	Me
8	H	H	Me

Figure 1. Structure of nobiletin (**1**), sudachitin (**2**) and their demethyl derivatives **3**–**8**.



Scheme 1. Synthesis of sudachitin (**2**).

As shown in Scheme 1, synthesis of sudachitin (**2**) was commenced with acetophenone **9**, which we have previously synthesized on a large scale.[3] Upon treatment of **9** with LiCl, regioselective demethylation reaction occurred at the 4-methoxy group to provide **10**.[9] Further treatment with benzyl bromide and K_2CO_3 resulted in regioselective incorporation of the benzyl group at the 4-hydroxy group to afford **11**. In the presence of LiHMDS, condensation reaction of acetophenone **11** and

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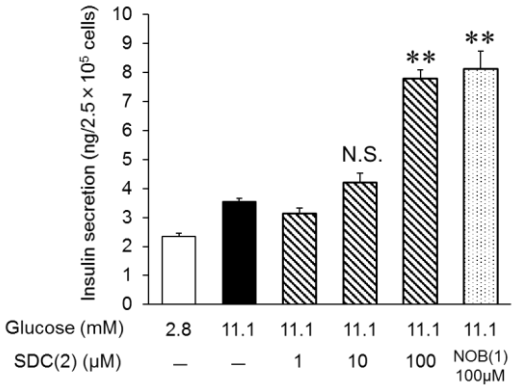
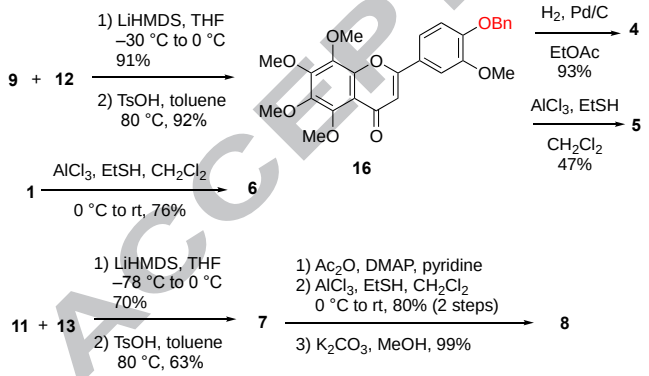
acylbenzotriazole **12**,[10] which was readily obtained from 4-hydroxy-3-methoxybenzoic acid and benzotriazole,[11] proceeded smoothly to give **14**. Without purification, acidic treatment of **14**



resulted in simultaneous cyclization and dehydration in one pot with concomitant cleavage of the benzyl ether to afford **3**. After acetylation of the phenol groups of **3**, removal of the methyl group was carried out by means of Node's protocol.[12] Treatment with EtSH in the presence of $AlCl_3$ resulted in regioselective removal of the methyl group to afford **15**. In this reaction, chelation of the Lewis acid by the carbonyl group played a key role in the regioselectivity and reactivity.[9] Finally, ammonolysis of the acetate provided **2**. All spectral data (^{1}H NMR, ^{13}C NMR and HRMS) were in full agreement with reported values.

With **2** in hand, we next turned our attention to the synthesis of the other desmethyl nobiletin derivatives as shown in Scheme 2. Firstly, 4'-desmethyl derivatives **4** and **5** were synthesized by condensation of **9** and **12** in the same manner as described for the synthesis of **2** and **3**. Selective deprotection of the benzyl ether of **16** by hydrogenation provided **4** and simultaneous removal of the benzyl and 5-methyl groups was performed by treatment with EtSH and $AlCl_3$ to give **5**. Compound **6** was readily obtained from **1** by applying our methodology. Condensation of **11** and **13** provided **7**, which was readily converted to **8** by similar treatment to that employed for **2**.



Scheme 2. Synthesis of demethyl nobiletin derivatives **4**–**8**.

Next, we compared the biological activity of **1** and **2** to enhance insulin secretion from rat β-cell line INS-1D. Compound **2** significantly enhanced glucose-induced insulin secretion at 100 μM, but not at 10 μM. Since we previously showed that **1** significantly promotes glucose-induced insulin secretion at 10 μM,[13] **2** seems to be slightly less potent than **1**, as shown in Figure 2.[14]

Figure 2. Effect of sudachitin (SDC) **2** on glucose-induced insulin secretion in INS-1D cells. The effect of nobiletin (NOB) **1** (100 μM) is also shown for comparison.

Compound **2** is known to inhibit cAMP-specific phosphodiesterase (PDE). The intracellular second messenger cAMP directly modulates protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac), which regulate various cellular signal transduction pathways. cAMP is catabolized by PDE, resulting in down-regulation of cAMP/PKA and cAMP/Epac signaling. Hence, we used an established assay to evaluate the inhibitory effects of **2** and its derivatives **3**–**8** on PDE activity, with nobiletin (**1**) as a positive control.[15, 16] We found that **2** and its derivatives inhibited PDE activity, with the rank order of **2** = **3** > **5** > **1** > **8** >> **4** > **6** > **7** at the concentration of 10 μM, as shown in Figure 3.

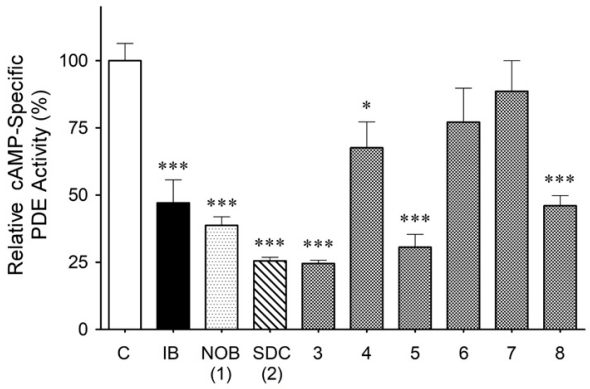


Figure 3. Inhibitory effects of polymethylated flavones on PDE activity.[16]

In conclusion, we have developed efficient and flexible methodology for the synthesis of methylated flavone derivatives. Free hydroxyl group(s) in these compounds should be suitable for incorporation of various probes moieties, which would be useful for chemical-biology studies.[2b] Detailed evaluation of the biological activities and biosynthetic route to **2** from **1** are in progress.

Acknowledgments

We thank Dr. Kazuo Okamoto, CEO of Ushio ChemiX Corporation for kindly providing acetophenone **9** (synthetic intermediate for nobiletin). This work was financially supported by MEXT/JSPS KAKENHI Grant Numbers JP17H03973 and JP17K15424, Grants-in-Aid for Scientific Research on Priority Areas JP16H01160 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, and the Platform Project for Supporting in Drug Discovery and Life Science Research (Platform for Drug Discovery, Informatics and Structural Life Science) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT).

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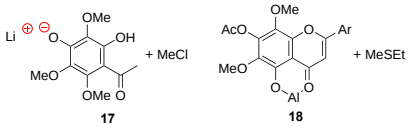
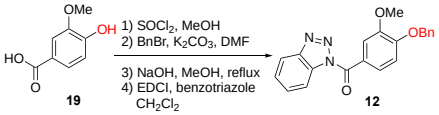
9. Regioselective demethylation reaction of **9** and **3** proceeded through the intermediates **17** and **18**.



17 18

10. Synthesis of **12** from **19** was performed as follows, similarly to the preparation of **13**.



19 12

66% (4 steps)

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14. INS-1D cells were incubated for 1 h in HEPES-buffered Krebs solution containing 2.8 or 11.1 mM glucose in the absence or presence of the indicated concentrations of sudachitin (**2**) and nobiletin (**1**). Each value is the mean ± SEM of eight independent experiments. $^{**}P < 0.01$ vs. 11.1 mM glucose without any addition.
15. Nagase, H.; Yamakuni, T.; Matsuzaki, K.; Maruyama, Y.; Kasahara, J.; Hinohara, Y.; Kondo, S.; Mimaki, Y.; Sashida, Y.; Tank, AW.; Fukunaga, K.; Ohizumi, Y. *Biochemistry* **2005**, *44*, 13683–13691.
16. The assays were performed using a cyclic nucleotide PDE assay kit (Enzo Life Sciences) according to the manufacturer's protocol. The 3',5'-cAMP substrate was cleaved by cAMP-specific PDE, resulting in the release of 5'-AMP. The 5'-nucleotidase cleaved the released 5'-AMP to nucleoside and phosphate. Phosphate was determined with a colorimetric assay using BIOMOL® GREEN reagent. The final concentration of DMSO was 1% (v/v) and the DMSO control was subtracted from the values of test compounds. The data were analyzed using a one-way ANOVA with Tukey's multiple-comparison test. C: control (absence of the test compounds and DMSO), IB: 3-isobutyl-1-methylxanthine (IBMX: PDE inhibitor, at a concentration of 40 μM), NOB (**1**): nobiletin (**1**), SDC (**2**): sudachitin (**2**). Histogram indicated the mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. control.

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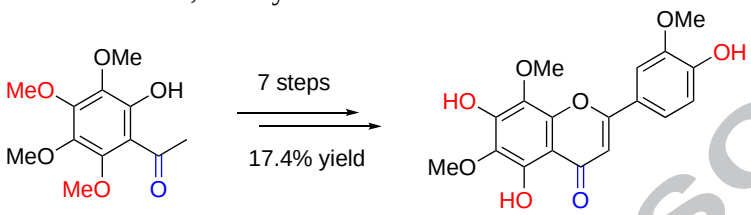

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