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A convenient and efficient approach to synthesize negletein from baicalin

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ABSTRACT

A simple two-step/one-pot and highly efficient strategy for the synthesis of negletein from naturally abundant and inexpensive baicalin has been developed. In this one-pot sequence, esterification of baicalin catalyzed by concentrated H_2SO_4 in methanol followed by the treatment with excess $NaBH_4$ afforded negletein in moderate yield. This method provides an additional approach for the synthesis of negletein.

Introduction

Flavonoids, which are ubiquitously distributed in many dietary plants, have received increasing attention because most of them display various beneficial effects on human health.[1–3] Baicalin (**1**) and its aglycone baicalein (**2**) (Scheme 1) are the major active ingredients of Chinese herb *Scutellaria baicalensis*.[4] Both of them have been demonstrated to possess various clinically relevant properties such as anti-oxidant, anti-cancer, anti-microbial, and anti-allergy activities.[4,5] However, both baicalin and baicalein suffer from poor aqueous solubility and low bioavailability, limiting their further potential clinical application.[6] Negletein (**3**), the methylated derivate at HO-C(7) position of baicalein, has similar biological effects with more favorable pharmacokinetic parameters and physicochemical properties including metabolic stability and aqueous solubility (Scheme 1).[7–9] Therefore, negletein represents a suitable substitute of baicalin/baicalein and an ideal starting point for further chemical optimization.[10]

However, the natural abundance of negletein is particularly low.[11] Toward this end, several approaches have been developed in order to obtain sufficient amount of negletein for further drug discovery. As shown in Scheme 2, starting from baicalein which was the deglycosylated product of baicalin, peracetylization was performed in Ac_2O and AcONa to afford derivative **4**. Selective deacetylation and then alkylation of HO-C(7) of compound **4** with methyl iodide provided corresponding derivative **5**. Deacetylation of compound **5** afforded negletein.[12] Kawabata et al. obtained negletein concisely through methylation of baicalein with methyl iodide and lithium carbonate in a decent yield of 72%.[13] Waghmode et al. utilized methylation of baicalein with MeI, K_2CO_3, and pyridine in acetone to yield 5,6,7-trimethoxyflavone (**6**). Selective didemethylation of **6** was observed at the 5, 6-position in the presence of a protic acid and a catalytic amount of phase-transfer catalyst (Aliquat-336) to afford negletein.[14] Righi et al. carried out the synthesis of negletein outlined in Scheme 3.[15] Methylation of HO-C(7) of crysin (**7**) provided compound **8**. Bromination of **8** with tetrabutylammonium tribromide (TBATB) afforded a mixture of **9** and **10**. The mixture was submitted to the usual methanolysis step to provide **11**. Demethylation of MeO-C(6) of **11** ran smoothly to give the desired product negletein.[15]

Although these above mentioned approaches can be used to successfully synthesize negletein, they have limited applicability due to the complex synthetic routes, expensive starting materials or difficult purification. For example, the first and the fourth methods need additional protection and deprotection manipulation steps. In addition, the intermediate **8** in the fourth method and negletein in the third method are not easy to be obtained because selective methylation is difficult to be manipulated, resulting in potential complicated purification. Therefore, development of an additional effective approach to synthesize abundant negletein for clinical validation and chemical modification is necessary.

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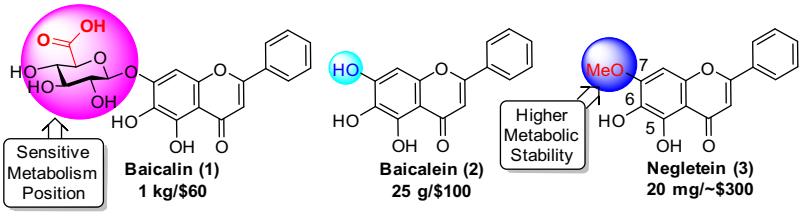
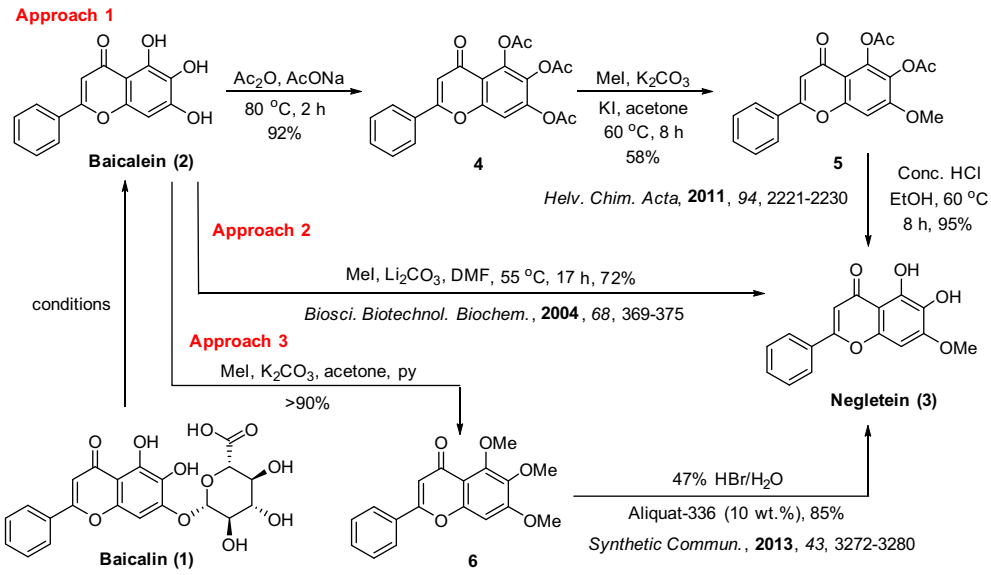
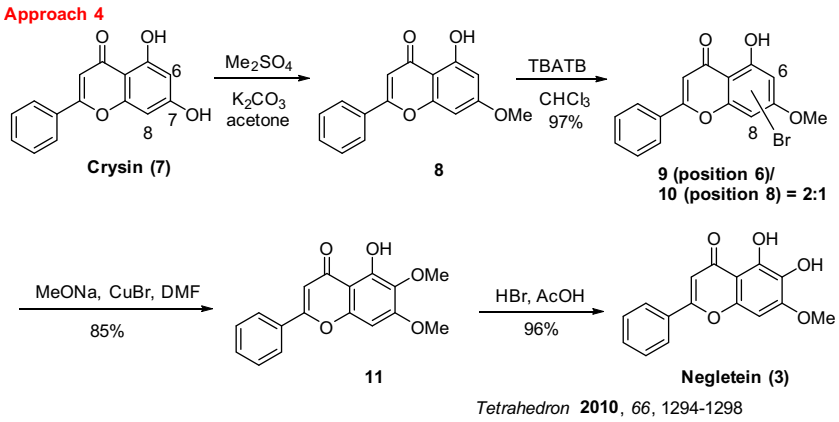


Scheme 1. Chemical structures of baicalin (**1**), baicalein (**2**), and negletein (**3**).



Scheme 2. Three approaches for synthesis of negletein from baicalein.

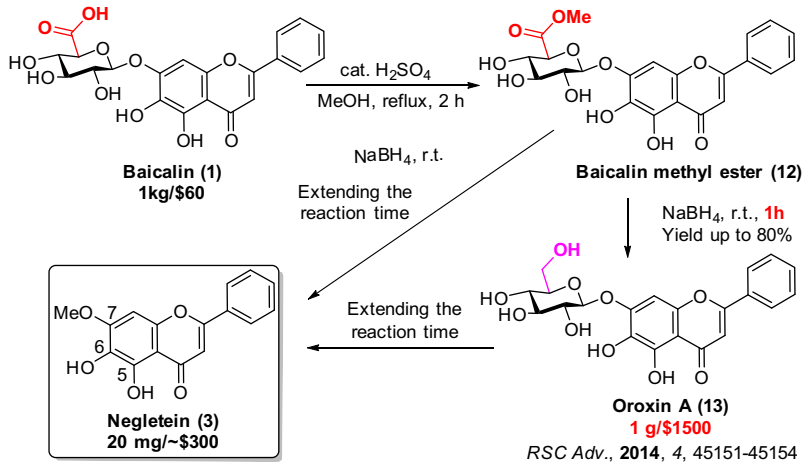


Scheme 3. Synthesis of negletein from crysin.

Results and discussion

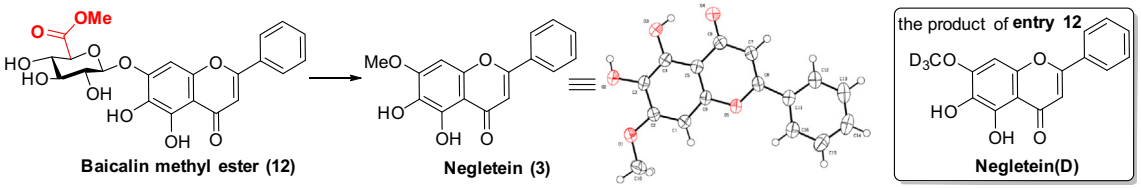
This new approach to synthesize negletein from baicalin was found during our reported process about the synthesis of oroxin A (**13**) which was shown in Scheme 4.[16] Baicalin was converted to baicalin methyl ester (**12**) in the presence of the catalytic amount of concentrated H_2SO_4 in the solution of methanol. The key intermediate (**12**) was then directly reacted with $NaBH_4$ in 1 h to afford oroxin A in a very high yield without further purification. To our surprise, when we extended the reaction time from 1 h to 12 h, two major components including oroxin A were detected by analytical TLC (thin-layer chromatography). The other major component was then purified (~11% yield) and structural characterization revealed this side product to be negletein. The chemical structure was further confirmed by single-crystal X-ray structural analysis.[17] To our best knowledge, this methylation is seldom reported. Based on the preliminary result, we envisioned that it might provide a new approach to synthesize negletein. In order to obtain abundant negletein for further chemical modification, various conditions were investigated.

As depicted in Table 1, we extended the reaction time from 12 h to 24 h, and the reaction proceeded smoothly to give negletein in 32% yield (entry 3). Notably, oroxin A also can be detected in the reaction mixture, indicating that we could extend the reaction time



Scheme 4. Reagents and conditions: (a) cat. H_2SO_4, MeOH, reflux; (b) $NaBH_4$, 0 °C to 25 °C.

Table 1
Optimization of the $NaBH_4$ reaction condition



Entry	$NaBH_4$ (equiv)	Solvent	T (°C)	Time (h)	Yield[a] (%)
1[b]	10.0	MeOH	0–25	1	0
2[b]	10.0	MeOH	25	12	11
3[b]	10.0	MeOH	25	24	32
4[b]	10.0	MeOH	25	48	51
5[b]	6.0	MeOH	25	48	32
6[b]	3.0	MeOH	25	48	8
7[c]	10.0	MeOH	25	48	50
8[d]	10.0	MeOH	25	48	48
9[e]	10.0	MeOH	25	48	40
10[f]	10.0	EtOH	25	48	0
11[g]	10.0	THF	25	48	0
12[h]	10.0	CD_3OD	25	48	52

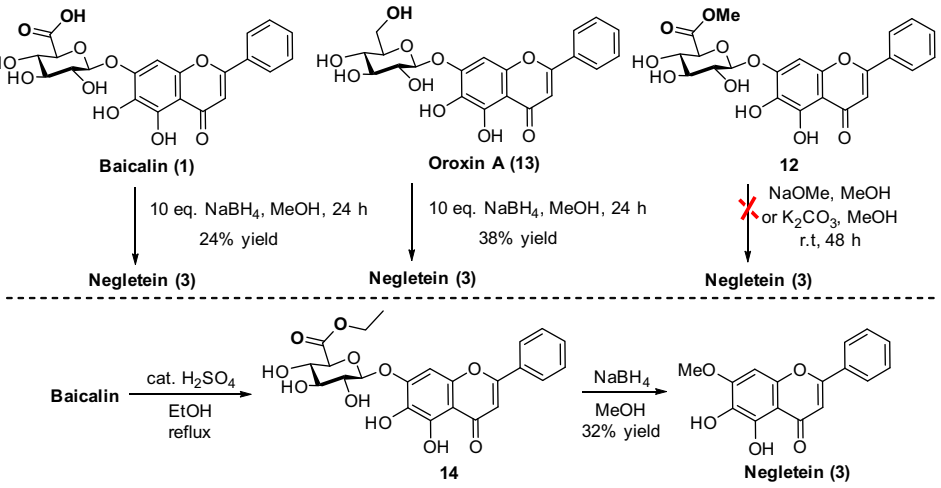
[a] Isolated yield.
[b] **12** (0.22 mmol), MeOH (10 mL).
[c] Large scale, **1** (5.6 mmol), cat. H_2SO_4, and MeOH (200 mL) in two-steps/one-pot.
[d] **1** (11.2 mmol), cat. H_2SO_4, and MeOH (400 mL).
[e] **1** (45 mmol), cat. H_2SO_4, and MeOH (1000 mL).
[f] **12** (0.22 mmol), EtOH (10 mL).
[g] **12** (0.22 mmol), THF (tetrahydrofuran, 10 mL).
[h] **12** (0.10 mmol), CD_3OD (2 mL).

to increase the conversion. Performing the reaction for 48 h resulted in full conversion and the desired product was obtained in 51% yield (entry 4). The optimization on the reaction condition revealed that the decrease in the amount of $NaBH_4$ had a dramatic effect on the yield (entries 5 and 6). We next carried out the reaction on gram scale from the starting material of baicalin and the results demonstrated the practicability of this methodology (entries 7–9). The desired product was obtained up to 40% yield even performing the reaction on 20 g scale (see Figs. S1–S12).

Having the optimal reaction condition, we then began the study to understand the reaction mechanism. First, we realized that with EtOH or THF as solvent, the reaction did not afford the desired product (entries 10 and 11). We then used K_2CO_3 or NaOMe as the substitute of $NaBH_4$ for this reaction. However, no desired product was observed (Scheme 5). These results indicated that $NaBH_4$ was an important and enabling reagent for this reaction. Next we intended to make clear whether the methoxyl moiety of negletein originated from glucuronic acid methyl ester or methanol.[18] To address these issues, baicalin or oroxin A without methoxyl group was directly subjected to $NaBH_4$ in the solution of MeOH for 24 h. These two starting materials smoothly generated negletein in the yield of 24% and 38%, respectively. We further used baicalin ethyl ester (**14**) with $NaBH_4$ in the solvent of MeOH. Intriguingly, not baicalein-7-ethylether but negletein was observed. We next used CD_3OD as the solvent and the desired product negletein(D) was obtained as expected (entry 12). These results demonstrated that the methoxy moiety of negletein came from methanol.

Conclusions

Herein, we present a simple two-step/one-pot chemical synthesis of negletein (**3**) using cheap starting material baicalin (1 kg/$60).



Scheme 5. Synthesis of negletein from baicalin or oroxin A or baicalin ethyl ester (**14**).

This method utilizes esterification of baicalin (**1**) catalyzed by concentrated H_2SO_4 in methanol and the consequent direct treatment with $NaBH_4$ to access negletein in a moderate yield. Our optimized process for the second step was primarily achieved by using $NaBH_4$ as reactant in the solvent of MeOH at 25 °C for 48 h to afford negletein in 50% yield. Our mechanism of investigation revealed that this procedure was accomplished by deglycosylation of baicalin and concomitant methylation by methanol in the presence of $NaBH_4$. The synthetic strategy developed in this work provided an additional facile approach for the synthesis of negletein for clinical validation and further chemical modification.

Experimental section

All commercially available starting materials and solvents were reagent grade, and used without further purification. Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. Preparative column chromatography was performed using silica gel 60, particle size 0.063–0.200 mm (70–230 mesh, flash). Analytical TLC was carried out employing silica gel 60 F254 plates (Merck, Darmstadt). Visualization of the developed chromatograms was performed with detection by UV (254 nm). NMR spectra were recorded on a Bruker-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal reference. Chemical shifts were expressed in ppm, and J values were given in Hz. High-resolution mass spectra (HRMS) were obtained from Thermo Fisher Scientific Exactive Plus mass spectrometer.

Synthesis of negletein (3)

The key intermediate baicalin methyl ester (**12**)[16] (0.1 g, 0.22 mmol) was suspended in methanol (10 mL) and cooled to 0 °C. Then $NaBH_4$ (46 mg, 1.22 mmol) was added portionwise. The reaction mixture was stirred at 25 °C for 48 h and then quenched with 10 mL of 1 N HCl (aq). The solution was evaporated to obtain the crude product. The residue was diluted with CH_2Cl_2/MeOH (10:1, 40 mL) and extracted with H_2O (40 mL). The organic layer was then washed with brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated to give the crude product. This residue was purified with silica gel column (CH_2Cl_2/MeOH = 20:1) to provide negletein (32 mg, 51%) as a yellow solid (mp 222–223 °C). ^{1}H NMR (400 MHz, DMSO-d_6): 12.50 (s, 1H), 8.77 (s, 1H), 8.06 (d, J = 7.1 Hz, 2H), 7.58 (t, J = 8.0 Hz, 3H), 6.96 (s, 1H), 6.92 (s, 1H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): 182.3, 163.1, 154.6, 149.8, 146.1, 131.9, 130.9, 130.1, 129.1, 126.3, 105.3, 104.7, 91.3, 56.3. HRMS (ESI) calcd for $C_{16}H_{11}O_5$ 283.0612 $(M-H)^-$, found 283.0611. Data agreed with those reported in the literature.[12–15]

One-pot large-scale synthesis of negletein (3)

To the solution of baicalin (20.0 g, 44.8 mmol) in methanol (400 mL) was added a catalytic amount of sulfuric acid (0.1 mL), and the mixture was heated at reflux temperature for 2 h. The mixture was added to methanol (600 mL) and cooled to 0 °C and then $NaBH_4$ (17.0 g, 448 mmol) was added portionwise in 2 h. After the addition was complete, the mixture was stirred for an additional 48 h at 25 °C and then quenched with 600 mL of 1 N HCl (aq). The solution was evaporated to obtain the brown suspension. The residue was diluted with CH_2Cl_2/MeOH (10:1, 1000 mL) and extracted with H_2O (400 mL). The organic layer was then washed with brine (200 mL), dried over anhydrous Na_2SO_4, and concentrated to give the crude product. This residue was purified with silica gel column (CH_2Cl_2/MeOH = 20:1) to afford the crude product, which was washed with EtOAc (50 mL) to give negletein (5.1 g, 40%) as a yellow solid. The structural characterization data are the same as those described above.

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Supplementary data

Supplementary data (photographic guide for the synthesis of negletein (20 g scale), copies of ^{1}H and ^{13}C NMR spectra of negletein, copies of ^{1}H NMR spectra of negletein(D), and HRMS spectra of negletein(D)) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.03.085.

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