Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 79 (10) 1205–1212 (2014)
JSCS–4658
UDC 577.175.73+547.918+547.563.13'023+
542.913+66.094.414+66.095.12:61(510)
Original scientific paper

Improved synthesis of gastrodin, a bioactive component of a traditional Chinese medicine

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(Received 11 October 2013, revised 23 March, accepted 24 March 2014)

Abstract: Highly practical, four-step synthesis of gastrodin was developed using penta-*O*-acetyl-*β*-D-glucopyranose and *p*-cresol as glycosyl donor and glycosyl acceptor, respectively, in 58.1 % overall yield. As the initial step, the penta-*O*-acetyl-*β*-D-glucopyranose was treated with *p*-cresol in the presence of $BF_3 \cdot Et_2O$ as catalyst to generate 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D--glucopyranoside in 76.3 % yield. Further, this product was subjected to radical bromination with *N*-bromosuccinimide (NBS) to provide 4-(bromomethyl)phenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside in 91 % yield. Subsequently, reaction of 4-(bromomethyl)phenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside with a solution of acetone and saturated aqueous sodium bicarbonate led to 4-(hydroxymethyl)phenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside in 93 % yield. Finally, global deprotection of 4-(hydroxymethyl)phenyl 2,3,4,6-tetra-*O*--acetyl-*β*-D-glucopyranoside under Zemplen conditions furnished gastrodin in 90 % yield. Compared to the previously reported methods, this protocol has the advantages of operational simplicity, chromatography-free separation, high overall yield, inexpensive and common reagents as well as less waste pollutants, rendering it an alternative suitable for industrial production.

Keyword: gastrodin; glycosylation; penta-*O*-acetyl-*β*-D-glucopyranose; radical bromination.

INTRODUCTION

Chemically known as 4-(hydroxymethyl)phenyl *β*-D-glucopyranoside, gastrodin is believed to be an important bioactive component of the famous Chinese herb *Gastrodia elata* B1, a well-known natural calcium channel blocker. Therapeutically, it has long been extensively used in China for the treatment of cardiovascular and cerebrovascular diseases, such as hypertension, stroke, migraine, dementia and hemiplegia.[1,2] In addition to its therapeutic functions in cardiovascular and cerebrovascular diseases, gastrodin has comprehensive pharmaco-

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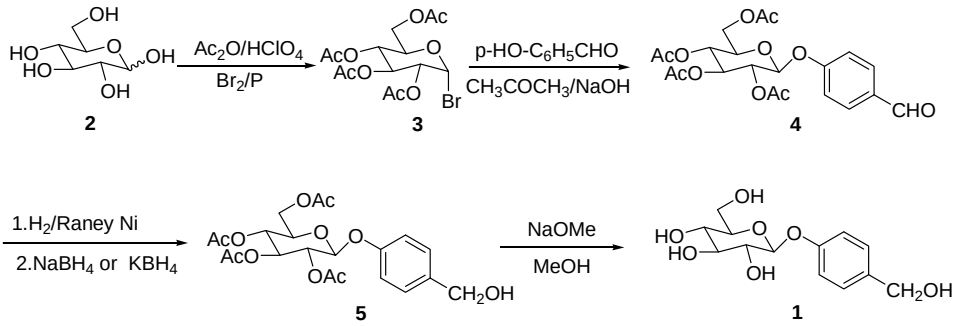
doi: 10.2298/JSC131011026L

logical profiles, including hypoxia tolerance, neuroprotective and anticonvulsant effect,[3–5] antioxidant and radical scavenger,[6] protection against cardiac hypertrophy and fibrosis[7] as well as an anti-myocardial ischemia effect in MI rabbits.[8] Recent studies have linked gastrodin with suppressing the inflammatory response in septic cardiac dysfunction[9] and stimulating anticancer immune response as well as repressing transplanted H22 hepatic ascetic tumour cell growth.[10]

Conventionally, gastrodin was extracted from *G. elata* flower,[11–13] which is a time-consuming and expensive process. In addition, this preparation procedure was challenged by the extremely low content (0.025 %) in the rhizome as well as the ever-increasing shortage of *G. elata* flower due to over exploitation. To circumvent these problems associated with the extraction of gastrodin from *G. elata* flower, a chemical synthesis of gastrodin was developed (Scheme 1),[14] in which 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide **3** was employed as a glycosyl donor. As shown in Scheme 1, this preparation of **3** involves the employment of bromine and red phosphorus in the presence of perchloric acid as catalyst, thereby generating a large volume of highly toxic and harmful bromine and phosphorus-containing waste pollutants that are detrimental to the environment and human health, and therefore raising additional safety concerns especially when handling on the industrial scale.



Scheme 1. Reported synthesis of gastrodin (**1**).

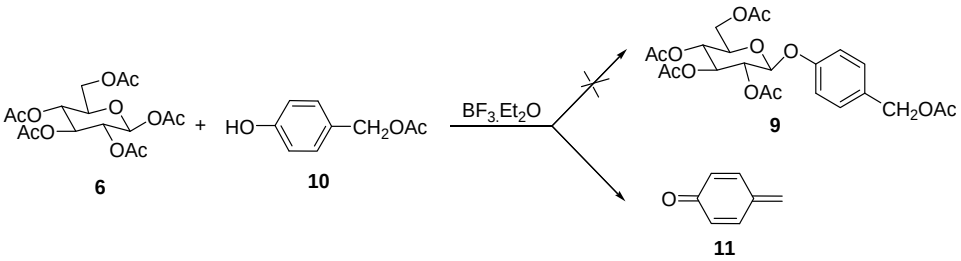
Although many new convenient and efficient alternatives for the synthesis of **3** in the absence of bromine and red phosphorus are available nowadays,[15] the moisture and heat-labile **3** led to the subsequent glycosylation in poor yield under the aqueous conditions. Moreover, conversion of 4-formylphenyl 2,3,4,6-tetra-*O*--acetyl-*β*-D-glucopyranoside **4** to 4-hydroxyphenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D--glucopyranoside **5** by either catalytic hydrogenation over Raney Ni at high pressure or chemical reduction with potassium (sodium) borohydride in methanol confers limited value for the large scale production of gastrodin due to the use of relatively expensive catalysts and reagents.

Recently, a laboratory-scale preparation of gastrodin was realized *via* biotransformation.[16–18] Unfortunately, preparation was seriously impaired by several drawbacks such as difficult strain development and cultivation, frequent strain variation, *i.e.*, large volumes of fermentation broth, a longer reaction time and extremely low yield, suggesting that it is still far removed from its commercial production *via* the biotransformation strategy.

In this context, the development of a chemical synthesis of gastrodin superior to the existing procedures is highly desired. Herein, a novel and efficient strategy for the chemical synthesis of gastrodin **1** using penta-*O*-acetyl-β-D-glucopyranose **6** and *p*-cresol **12** as a glycosyl donor and glycosyl acceptor, respectively, thereby avoiding many disadvantages inevitable in the previously reported procedures, is presented. The operational simplicity, cost-effectiveness and high overall yield of the procedure would make this new strategy suitable for the industrial production of gastrodin.

RESULTS AND DISCUSSION

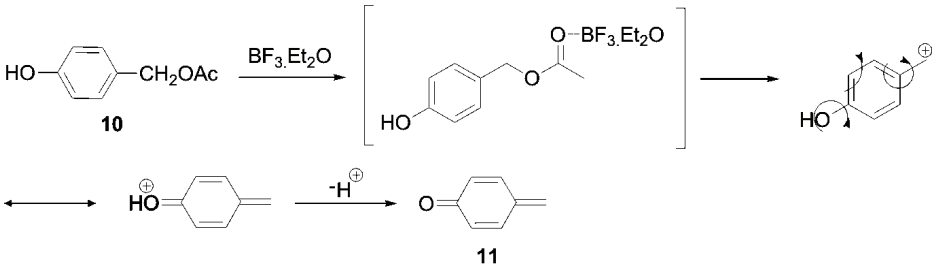
Initially, the synthesis of **1** according to Scheme 2 was attempted. Disappointedly, treatment of **6** with 4-hydroxybenzyl acetate **10**[19] in the presence of $BF_3{\cdot}Et_2O$ as catalyst led to the undesired compound **11** as a pink precipitate instead of the desired compound **9**. The suggested mechanism underlying the formation of compound **11** is depicted in Scheme 3.



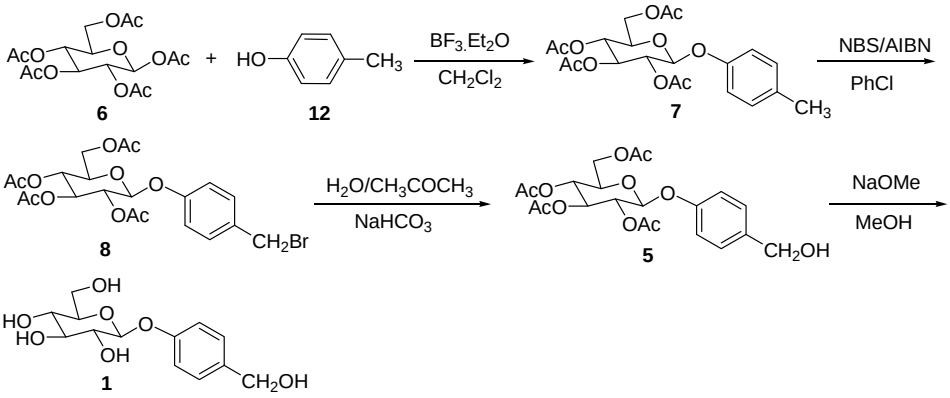
Scheme 2. Unsuccessful synthesis of gastrodin (**1**).

Frustrated with chemical synthesis of gastrodin **1** according to Scheme 2, another synthetic route was attempted, as shown in Scheme 4. As the first step, **6** was treated with **12** in the presence of $BF_3{\cdot}Et_2O$ as catalyst to generate 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **7** using a similar method as that described in the literature,[20] with some modifications, *i.e.*, the glycosylation was performed using $BF_3{\cdot}Et_2O$ alone as catalyst instead of the $BF_3{\cdot}Et_2O$–Et_3N combination and the time for the glycosylation was much shorter than in the reference. Additionally, product **7** was purified by crystallization from ethanol in 76.3 % yield and confirmed to be the β anomer by the coupling constant ($J_{1,2}$ = = 7.2 Hz) calculated from the ^{1}H-NMR spectrum of **7** (Supplementary material to

this paper). Subsequently, radical bromination of **7** was accomplished through reaction with *N*-bromosuccinimide (NBS) at 67 °C in the presence of azodiisobutyronitrile (AIBN) or benzoyl peroxide (BPO, dibenzoyl peroxide) as initiator. At first, CCl_4 was selected as the reaction solvent for the radical bromination of **7**, but the reaction did not proceed at all irrespective of whether AIBN or BPO was used as the initiator. This indicates that the choice of the reaction solvent is of crucial importance for the successful synthesis of 4-(bromomethyl)phenyl 2,3,4,6--tetra-*O*-acetyl-β-D-glucopyranoside **8**.



Scheme 3. Plausible mechanism for the formation of **11**.



Scheme 4. Practical synthesis of gastrodin (**1**).

To this end, a solvent screening was conducted among solvents commonly used in the radical bromination reaction, including CCl_4, *n*-hexane, cyclohexane, benzene, $CHCl_3$ and chlorobenzene, to determine the best solvent with respect to reaction yield. As a result, chlorobenzene was found to be the optimal one. Additionally, to test if the mole ratio of NBS to **7** and manner of NBS addition could affect the yield of **8**, a mole ratio titration was conducted and it was found that a molar ratio of NBS to **7** of 1.2:1 and the portion-wise addition of NBS gave the highest yield, presumably because it could significantly prevent the formation of the dibrominated side-product. Under these circumstances, **8** was obtained by reaction of **7** with NBS in 91% yield (the highest). Theoretically, the formation

of gastrodin **1** is achievable *via* direct hydrolysis of **8** under strong basic conditions, but separation of gastrodin **1** by extraction from aqueous mixture is difficult practice due to its strong hydrophilicity and insolubility in water-immiscible organic solvents. Consequently, a selective hydrolysis of **8** into **5** is advisable. Therefore, interaction of **8** with a mixture of acetone and saturated aqueous sodium bicarbonate solution at 50 °C gave rise to product **5**. Of special note is that the volume ratio of acetone to saturated aqueous sodium bicarbonate affects the reaction rate and yield. To determine the optimal ratio, a titration was performed and the results showed that a volume ratio of 4:1 of acetone to saturated aqueous sodium bicarbonate solution gave the best yield (93 %) after the usual aqueous work-up. Finally, global deprotection of **5** under Zemplen conditions (NaOMe/MeOH system) followed by recrystallization from methanol–chloroform (1:8, *V*/*V*) furnished gastrodin (**1**) as white crystals in 90 % yield.

EXPERIMENTAL

Materials and methods

Penta-*O*-acetyl-*β*-D-glucopyranose, *p*-cresol, $BF_3{\cdot}Et_2O$, NBS and AIBN were obtained from Sigma–Aldrich. Sodium methoxide was obtained from the Qingdao Justness Reagent Co. (China). All solvents were of reagent grade and used without further purification unless otherwise stated and deionised water was used. CH_2Cl_2 was dried with CaH_2 under reflux and freshly distilled prior to use.

Instrumentation

All the synthesized compounds were confirmed by spectral methods, ^{1}H-NMR and ^{13}C--NMR spectroscopy and HRMS (ESI). The ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Bruker Avance III400 spectrometer, operating at 400 MHz for protons and 100 MHz for carbons. 2D NMR techniques (^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC) were used for full assignment of the spectra. Thin layer chromatography was performed on silica gel plates (GF254, Qingdao Haiyang Chemical Plant, China), and detection was effected by UV irradiation and subsequent charring with 10 % sulphuric acid in ethanol followed by heating. The melting points were determined with a digital melting point apparatus (WRS-1B) without correction. The optical rotations were measured with JASCO P1030 polarimeter.

Physical, analytic and spectral data are given in the Supplementary material to this paper.

Synthesis of 4-methylphenyl 2,3,4,6-tetra-O-*acetyl*-β-*D-glucopyranoside (**7**)*. To a stirred solution of *p*-cresol (**12**, 27.0 g, 0.25 mol) and penta-*O*-acetyl-*β*-D-glucopyranose (**6**, 65.0 g, 0.16 mol) in 200 mL dry CH_2Cl_2 was added dropwise a solution of $BF_3{\cdot}Et_2O$ (0.25 mol, 29.8 mL) in 30 mL dry CH_2Cl_2 at 0 °C within 1 h. The reaction mixture was then stirred for 2 h at room temperature, neutralized with saturated aqueous sodium bicarbonate (200 mL) and extracted with CH_2Cl_2 (2×100 mL). The combined organic layer was washed with saturated aqueous NaCl and dried over anhydrous sodium sulphate. Then, the filtrate was concentrated under reduced pressure to give yellowish solid crude product that was recrystallized from 95 % ethanol to afford the desired compound **7** as white crystals. Yield: 76.3 %.

Synthesis of 4-(bromomethyl)phenyl 2,3,4,6-tetra-O-*acetyl*-β-*D-glucopyranoside (**8**)*. A suspension of 4-methylphenyl-2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside (**7**) (43.8 g, 0.1 moL) in 300 mL chlorobenzene was heated to 67 °C to obtain a clear solution followed by the addition of AIBN (0.42 g, 0.0024 mol). To the obtained clear solution, a small portion of a

suspension containing NBS (21.4 g, 0.12 mol) in 100 mL of chlorobenzene was added. The initiation of the reaction was easily indicated by orange colour and a 2 °C temperature increase in the reaction mixture. Subsequently, the remaining NBS suspension in chlorobenzene was added portionwise within 1 h while maintaining the temperature at 64–69 °C. The reaction mixture was stirred for another 1 h at 67 °C before cooling to 40 °C. The succinimide formed in the reaction was removed by filtration and washed twice with chlorobenzene and the chlorobenzene washings were combined with the filtrate. The combined filtrate was evaporated to dryness under vacuum to give the crude glucopyranoside (**8**), which was recrystallised from absolute ethanol to furnish 52.5 g of compound **8** as white needle-like crystals. Yield: 91 %.

Synthesis of 4-(hydroxymethyl)phenyl 2,3,4,6-tetra-O-*acetyl*-β-*D-glucopyranoside (**5**)*. To a stirred solution of 4-(bromomethyl)phenyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranoside (**8**) (22.70 g, 0.05 mol) in 200 mL acetone was added 50 mL of a saturated aqueous sodium bicarbonate solution, and then heated to 50 °C for 10 h. The reaction mixture was evaporated under vacuum to recover the acetone solvent and the remaining aqueous solution was extracted with CH_2Cl_2 (2×120 mL). The combined CH_2Cl_2 layers were washed with saturated aqueous NaCl (100 mL) and dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to give a white solid crude product that on recrystallization from absolute ethanol afforded 21.12 g of the desired compound **5** as white crystals. Yield: 93 %.

Synthesis of 4-(hydroxymethyl)phenyl β-*D-glucopyranoside (**1**)*. Sodium methoxide (200 μL, 1 M in MeOH) was added to a solution of **5** (9.92 g, 0.020 mol) in 100 mL dry methanol under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 5 h followed by neutralization with Amberlite IR 120(H^+). Then the reaction mixture was filtered, and the resulting filtrate was evaporated to dryness under vacuum. Finally, recrystallization of the crude product from methanol–chloroform (1:8, *V*/*V*) yielded 5.21 g of the desired compound **1** as white crystals. Yield: 90 %

CONCLUSIONS

In summary, a novel efficient, and practical protocol for gastrodin synthesis in four sequential chemical steps was developed, in which penta-*O*-acetyl-*β*-D--glucopyranose was used as the glycosyl donor instead of 2,3,4,6-tetra-*O*-acetyl--*α*-D-glucopyranosyl bromide, in an overall yield of 58.1 %. Moreover, the presented protocol has the advantages over previous ones in terms of operational simplicity, commercially available and inexpensive reagents, easy separation and purification procedure dispensing with tedious and laborious chromatography, which render this protocol possibly suitable for the commercial production of gastrodin.

SUPPLEMENTARY MATERIAL

Physical, analytic and spectral data are available electronically from http:// //www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgments. This work was supported by a grant from the Natural Science Foundation of Shandong Province (No. ZR2011BL003) and a grant from the Start-up Foundation of high talents in Qingdao Agricultural University, Qingdao, P. R.China (No. 630708).

ИЗВОД

УНАПРЕЂЕНА СИНТЕЗА ГАСТРОДИНА, БИОЛОШКИ АКТИВНЕ СУПСТАНЦЕ ИЗ ТРАДИЦИОНАЛНЕ КИНЕСКЕ МЕДИЦИНЕ

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Развијен је практичан поступак синтезе гастродина, употребом пента-*O*-ацетил-*β*-D-глукопиранозе и *p*-крезола као гликозил донора и акцептора, редом, у четири реакциона корака у укупном приносу од 58 %. Реакцијом између пента-*O*-ацетил-*β*-D-глукопиранозе и *p*-крезола у присуству $BF_3{\cdot}Et_2O$ као катализатора добијен је (4-метилфенил)-2,3,4,6-тетра-*O*-ацетил-*β*-D-глукопиранозид у приносу од 76,3 %. Производ је бромован помоћу NBS и добијен је [4-(бромметил)фенил]-2,3,4,6-тетра-*O*-ацетил-*β*-D-глукопиранозид (принос 91 %) који је помоћу засићеног воденог раствора натријум-бикарбоната, реакцијом у ацетону, преведен у [4-(хидроксиметил)фенил]-2,3,4,6-тетра-*O*-ацетил-*β*-D-глукопиранозид (принос 93 %). Даљом реакцијом под Земпленовим условима добијен је гастродин (принос 90 %). У поређењу са раније описаним поступцима, нов протокол је једноставнији, омогућава лакше пречишћавање компоненти хроматографијом, висок укупан принос, употребу приступачних реагенаса, даје мање опасних отпадних компоненти и погодан је за индустријску производњу.

(Примљено 11. октобра 2013, ревидирано 23. марта, прихваћено 24. марта 2014)

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