OL Organic Letters

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Selective Iodination Enables Anthocyanin Synthesis to Be More General

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Cite This: *Org. Lett.* 2023, 25, 2289−2293 Read Online

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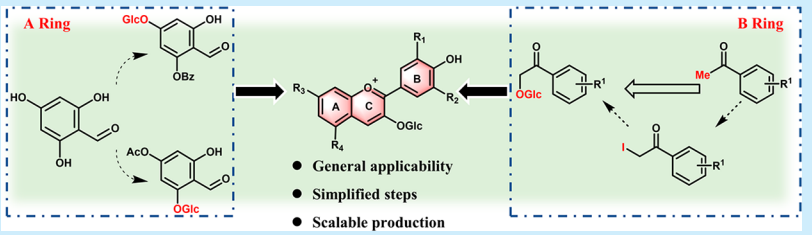


ABSTRACT: The current synthesis routes of anthocyanins are relatively complicated, which significantly hinders their development. We optimized the method by introducing a selective iodination reaction and also established a general scheme for preparing anthocyanin diglycosides. This method allows anthocyanin synthesis to require fewer steps and costs. Based on this, we prepared four common anthocyanins and two anthocyanin diglucosides and measured their antioxidant and anti-insulin resistance activities.

Anthocyanins are recognized as natural pigments with the C6−C3−C6 2-phenyl benzopyran cation.[1,2] They have been extensively investigated for their health benefits, such as cancer prevention, anti-inflammation, and antioxidation.[3,4] However, the targeted mechanisms of the health benefits of anthocyanin remain a challenge, due to the complex constituents of anthocyanin extracts. Over the years, the production of monomeric anthocyanin has been mainly done by the traditional method with solvent extraction using dark fruits and vegetables.[5−7] Production of anthocyanin with high purity remains a big challenge for industrialization.[8] In contrast, the chemical synthesis method has shown obvious advantages in achieving large-scale production and specific anthocyanin product application with high purity.[9] However, only a few chemical synthesis routes have been reported. The present methods for the chemical synthesis of anthocyanins are mainly performed by aldol condensation,[10,11] reduction of flavonoids by metals,[12,13] and biomimetic oxidation routes.[14] Referring to the structural uniqueness of anthocyanin, it is difficult to synthesize and modify, limiting its industrialization and application, particularly for anthocyanin diglucosides. It is critical to design a general and efficient route to synthesize various types of anthocyanins with lower cost and higher quality.[15]

The classical and common chemical synthesis of monomer anthocyanins is well-known as aldol condensation, which was first reported by Robinson et al.[11] In this method, acetophenone (B ring) and *o*-hydroxy benzaldehyde (A ring) are subjected to nucleophilic addition and dehydration by a cross-aldol condensation reaction in acidic conditions. As shown in Figure 1, the *ortho*-hydroxyl group of the A ring is

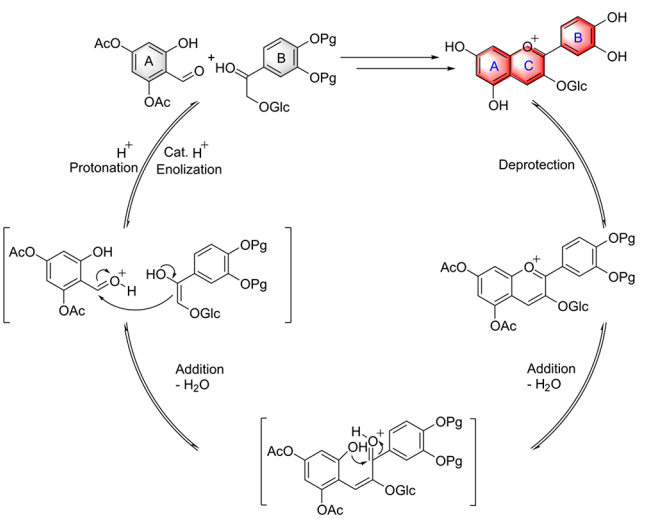


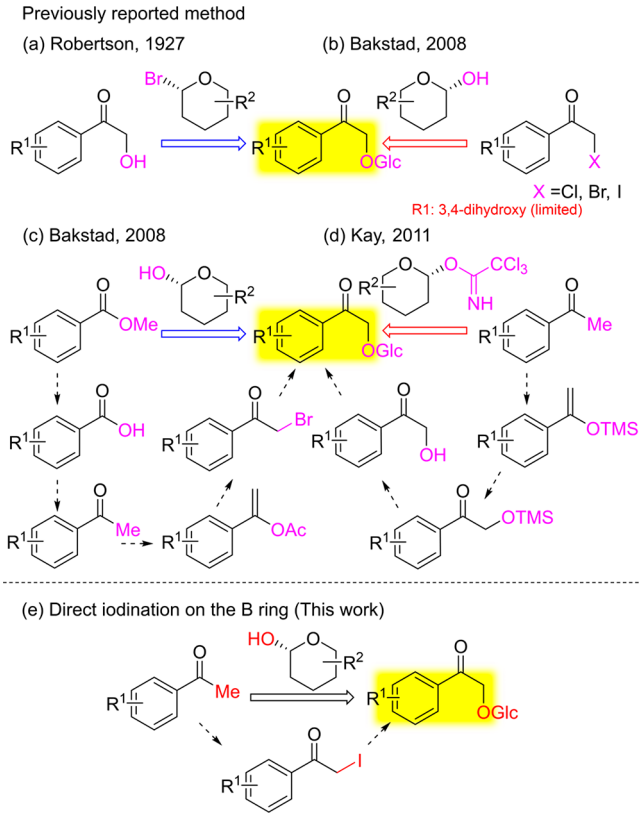
Figure 1. Mechanism of anthocyanin synthesis by aldol condensation.

Received: February 23, 2023
Published: March 22, 2023

https://doi.org/10.1021/acs.orglett.3c00597
Org. Lett. 2023, 25, 2289−2293

added to the carbonyl group of the B ring and then eliminated to form the oxonium ion pyran ring of the C ring. Unfortunately, although the final cyclization reaction is relatively mature, the intermediate preparation still cannot achieve universal efficiency. As shown in Scheme 1a, the

Scheme 1. General Methods to Obtain the B Ring



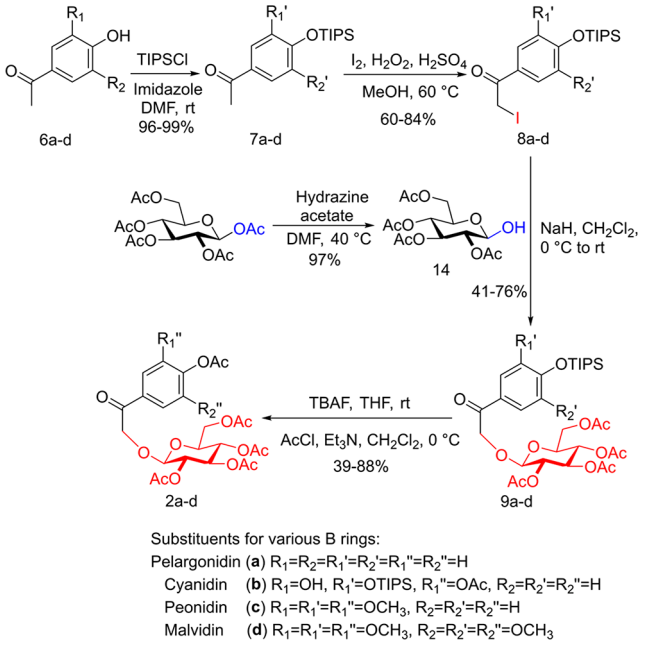
Koenigs−Knorr reaction required extreme drying conditions to synthesize the B ring with only 8% yield, following the method of Robertson et al. However, the yield of the C ring formation step is relatively low, so the preparation efficiency of the B ring is very important for the large-scale synthesis of anthocyanins. Based on this, Bakstad et al.[16] reported an improved preparation method for the B ring. For the raw material of 2-chloro-1-phenylethanone, after protecting the hydroxyl group, the halogen was exchanged with sodium iodide and then formed a glycosidic bond with the sugar oxyanion (Scheme 1b). On the other hand, more complicated operations were required for halogen-free raw materials. The hydroxybenzoate was finally converted into an α-halogenated acetophenone structure through a series of operations, which formed a glycosidic bond (Scheme 1c). Freitas et al.[17,18] also similarly synthesized anthocyanin metabolites. Kay et al.[19] proposed a different method when they synthesized isotope-labeled anthocyanin. As shown in Scheme 1d, the silyl enol ether was generated by the reaction of TMSCl, and acetophenone was treated with mCPBA. Then α-hydroxy acetophenone was obtained after in situ hydrolysis, which was further coupled with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate. At the same time, it is noteworthy that the synthesis of 5- or 7-glucose-substituted anthocyanin remains under-reported. Sperry et al.[20] developed a synthetic method to connect glucose to the para position of phloroglucinol aldehyde for nudicaulins synthesis. To selectively protect the *ortho*-hydroxyl, benzoyl chloride was employed and then glycosylated by glucopyranosyl bromide. However, the chemical synthesis of anthocyanin 5-glucoside has not been clearly reported. Dangles et al.[21] tried to glycosylate the *ortho-para*-hydroxyl of phloroglucinol aldehyde together when preparing 3-deoxyanthoanidins, but the polarity of the two end products, 5- and 7-glucose-substituted 3-deoxyanthoanidins, was too close to be easily separated again. Despite so many attempts at the chemical synthesis of anthocyanins, it remains a challenge to establish a general and efficient anthocyanin synthesis method. Herein, this study established a highly compatible synthetic route for synthesizing the A and B rings separately. The B ring was synthesized via methyl iodination of acetophenone in a greener and lower-cost manner (Scheme 1e). The 3,5- and 3,7-substituted anthocyanin diglucosides were successfully synthesized by introducing glucose at the ortho or para of the A ring aldehyde group.

Inspired by the selectivity of the halogenation reaction,[22−24] the direct substitution of halogens on B ring raw materials would simplify the preparation and expand the raw materials required for different anthocyanins. Through the retrosynthetic synthesis analysis of the B-ring, the key is to select the appropriate halogen in the α position to replace methyl without affecting other positions. It is well-known that different halogens have different reactivity with sodium alkoxide. We chose iodine to perform the halogenation reaction because it has higher leaving ability and product stability, resulting in the best reaction yield with sodium alkoxide and a typically lower risk of multisubstitution.[25] Additionally, our work demonstrated that the iodization process had excellent selectivity because no byproducts were iodized at any other positions. As for the hydroxyl-protecting group, we chose TIPSCl, which is easy to react with and remove. It was also found in the experiment that it did not affect our subsequent synthesis. Moreover, since TIPS is easy to remove, it can be replaced by acetyl groups before the cyclization reaction, thereby further improving the yield of targeted anthocyanins.

As shown in Scheme 2, the B rings of several differently substituted anthocyanins were manufactured utilizing *p*-acetyl phenyl analogs (**6a−d**) as starting materials. The TIPS protecting group is stable enough in strong bases to avoid undesirable byproducts in the following step and prevents iodine substitution. Therefore, in the first step, the hydroxyl groups in compounds **6a−d** were protected by reacting imidazole as the deacid reagent and TIPSCl in DMF at room temperature. Next, the reaction was stirred at 60 °C with methanol as solvent, followed by the addition of iodine and H_2O_2 and catalytic amounts of sulfuric acid. To our delight, the reaction produced compounds **8a−d** as a selective iodination product in high yield, which greatly reduced the tedious step. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucose was obtained by heating β-D-glucose pentaacetate in DMF solution with hydrazine acetate at 40 °C.[26] It was then made into a sodium salt with NaH at 0 °C and substituted with compounds **8a−d** to give compounds **9a−d** as a saccharified B ring. In the final stage of B ring formation, compounds **9a−d** and TBAF were reacted in THF at room temperature, and the intermediate product was reacted directly with AcCl and Et_3N in DCM solution at 0 °C to give B rings **2a−d** (Scheme 2). Replacing the protecting group at this step can avoid loss of yield after the final anthocyanin cyclization.

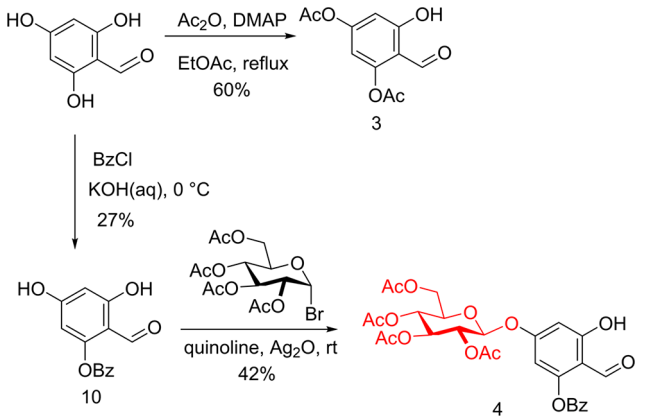
https://doi.org/10.1021/acs.orglett.3c00597
Org. Lett. 2023, 25, 2289−2293

Scheme 2. Synthesis of B Ring (2a−d)



Furthermore, we found that the chemical synthesis of common (3,5- and 3,7-) anthocyanin diglucosides in nature is almost unreported; therefore, we set out to design the synthetic routes for them. A ring synthesis of 3-glucose-substituted anthocyanins requires just 2,4,6-trihydroxy benzaldehyde with AC_2O and DMAP refluxed in ethyl acetate to yield compound **3** (Scheme 3). The A ring synthesis method

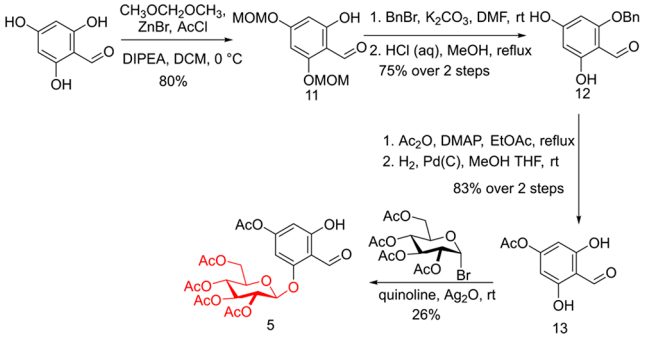
Scheme 3. Synthesis of 3 and 4



of 3,7-anthocyanin diglucoside was according to the report of Sperry et al.[20] 2,4,6-Trihydroxy benzaldehyde and BzCl were stirred in potassium hydroxide solution at 0 °C to obtain ortho-protected compound **10**, and then it was coupled with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl bromide in an Ag_2O suspension in quinoline at room temperature to give compound **4** (Scheme 3). To synthesize the 3,5-anthocyanin diglucoside, we initially tried a method that protects the para-position of 2′,4′,6′-trihydroxyacetophenone.[27] Unfortunately, there was no product when the raw material was trihydroxybenzaldehyde. The *ortho-para*-hydroxyl group was then protected with MOM and benzyl groups, respectively, before coupling glucopyranosyl bromide or even glucose trichloroimide ester. However, none of them produced the expected products due to steric hindrance. Finally, we chose to synthesize the para-monoprotected trihydroxybenzaldehyde by various protecting groups and then introduce glucose into the ortho position of the aldehyde group. To obtain compound **11** with only ortho exposure, $ZnBr_2$ was stirred in dimethoxymethane, and then AcCl was added dropwise to the stirred solution.

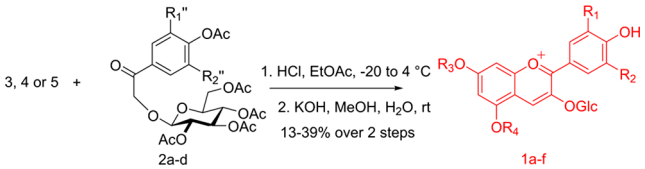
The solution was stirred at room temperature for another 2 h before being transferred to an ice-cold DCM solution containing predried 2,4,6-trihydroxy benzaldehyde and DIPEA. Compound **11** and K_2CO_3 were sequentially added dropwise to a stirred solution of BnBr in DMF and stirred for 6 h at room temperature. The 2 M HCl solution was refluxed in methanol for 3 h, and the MOM protection was removed to obtain compound **12**. It was refluxed with Ac_2O and DMAP in EtOAc and then reduced with hydrogen and Pd/C to obtain compound **13**. Compound **5** (Scheme 4) was then synthesized

Scheme 4. Synthesis of 5



by the same synthetic method as compound **4**. Finally, this method worked, and we successfully obtained the A ring with ortho substitution of glucose. After the A and B rings were prepared, anthocyanins were synthesized by passing dry HCl gas in EtOAc at −20 °C using the classical ring-forming conditions.[19] A ring **3** was reacted with B rings **2a−d** to obtain anthocyanins **1a−d**, respectively (Table 1). B ring **2b** was

Table 1. Synthesis of Anthocyanins 1a−f



Entry	Compound	Anthocyanin	B Ring	A Ring
1	**1a**	P3G	**2a**	**3**
2	**1b**	C3G	**2b**	**3**
3	**1c**	Pn3G	**2c**	**3**
4	**1d**	M3G	**2d**	**3**
5	**1e**	C-3,7-di-O-G	**2b**	**4**
6	**1f**	C-3,5-di-O-G	**2b**	**5**

reacted with A rings **4** and **5** to obtain 3,7-anthocyanin diglucoside **1e** and 3,5-anthocyanin diglucoside **1f**, respectively (Table 1). We also compared the physicochemical properties of **1b** (C3G) with native C3G (Figure S1), as well as the cytotoxicity, antioxidant activity, and anti-insulin resistance effects of **1a−f** (Figures S2 and S3). The results showed that our synthetic anthocyanins were safe, equivalent, and active.

https://doi.org/10.1021/acs.orglett.3c00597
Org. Lett. 2023, 25, 2289−2293

In summary, We have developed a general method for the preparation of the anthocyanin B ring by selective iodization from readily available starting materials. This strategy has proved to be universally applicable and can be scaled up. Glucosides were connected to the ortho or para of the A ring aldehyde group, respectively, which solved the problem of synthesizing two common anthocyanin diglycosides. Based on these advantages, high-quality anthocyanins can be obtained at a lower cost, which can promote related research to be better carried out and fully tap more possibilities. Furthermore, this method can also provide a lot of help in the chemical modification of anthocyanins.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its online Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.3c00597.

Experimental procedures, NMR spectra, spectroscopic data for new compounds, and activity data (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The current study is supported by the National Natural Science Foundation of China (No. U22A20546, 32172220, and 32272336) and the Guangdong Key Area Research and Development Program (No. 2022B0202040003). This work was also funded by the Youth Science and Technology Innovation Talent of Guangdong Tezhi Plan (No. 2019TQ05N770). The authors also thank the Outstanding Innovative Talents Cultivation Funded Programs for Doctoral Students of Jinan University (No. 2021CXB021).

■ ABBREVIATIONS

Glc, glucose; TIPSCl, triisopropyl chlorosilyl; MOM, methoxymethyl; P3G, pelargonidin-3-*O*-glucoside; C3G, cyanidin-3-*O*-glucoside; Pn3G, peonidin-3-*O*-glucoside; M3G, malvidin-3-*O*-glucoside; C3,7-di-*O*-G, cyanidin-3,7-*O*-diglucoside; C3,5-di-*O*-G, cyanidin-3,5-*O*-diglucoside; mCBPA, 3-chlorobenzenecarboperoxoic acid; DMAP, 4-dimethylaminopyridine

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