Synthesis of Metabolite of Resveratrol by Beauveria bassiana

Subjects: Chemistry, Medicinal

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Resveratrol is a well-known dietary polyphenol because it has a variety of beneficial biological activities. The fungus Beauveria bassiana is one of the most frequently used microorganisms for the biotransformation of polyphenols. Recently, resvebassianol A (2), a glycosylated metabolite of resveratrol by B. bassiana, was isolated and structurally elucidated. It was demonstrated to exhibit antioxidant, regenerative, and anti-inflammatory activities with no cytotoxicity. Here, we report the first total synthesis of resvebassianol A, 4'-O-β-(4'''-O-methylglucopyranosyl)resveratrol (2), and its regiomer, 3-O-β-(4 -O-methylglucopyranosyl)resveratrol (3). Key reactions include (i) the construction of a stilbene core via a novel Heck reaction of aryl halides and styrenes, and (ii) glycosylation with unnatural methylglucopyranosyl bromide. The glycosylation step was carefully optimized by varying the bases and solvents. Resveratrol metabolites 2 and 3 were obtained at 7.5% and 6.3% of the overall yield, respectively.

resveratrol resvebassianol A Beauveria bassiana metabolites glycoslyation

1. Introduction

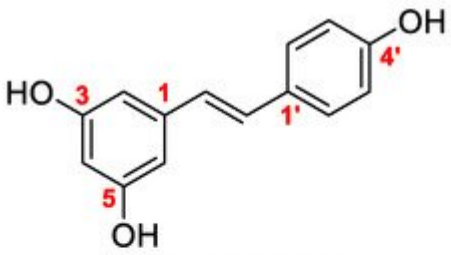
Resveratrol (**1**, *trans*-3,5,4-trihydroxystilbene) is an important dietary polyphenol and naturally occurring phytoalexin found in grapes, red wine, berries, peanuts, olive oil, etc. [1][2][3]. It is produced by plants in response to environmental stress and fungal attack through the induction of resveratrol synthetase [4][5]. Resveratrol was first isolated from the roots of the white hellebore lily (*Veratrum grandiflorum O. Loes*) in 1940 [6]. Most of the biological activities of resveratrol have been shown by its *trans* stilbene isomer, while the *cis* stilbene isomer also occurs naturally [7]. Resveratrol exerts numerous biological activities such as antioxidant, anti-infective, anti-inflammatory, anti-ischemic, cardioprotective, neuroprotective, anti-aging, anti-viral, anti-obesity, and anti-cancer effects [8][9][10][11][12][13][14][15][16][17][18]. Recently, it was revealed that its ability to activate various deacetylase enzymes (sirtuins) could be responsible for the various biological properties and delay aging [19][20].

Despite their pharmacological activities, various in vivo studies have shown that the potential of polyphenols is impaired by their insolubility in water, ultraviolet light instability, poor intestinal absorption, short half-life, rapid clearance, low bioavailability, and rapid metabolism [21][22]. The introduction of a glycosyl moiety on polyphenols not only helps to enhance the solubility of substrates but also reduces their toxicity, which ultimately increases the activity of biosynthetic intermediates [23]. Moreover, the sugar moiety of polyphenol glycosides might play a major role in their absorption, resulting in an acceptable concentration in the circulatory streams [24]. Polyphenols are subjected to enzymatic oxidation by polyphenol oxidases in plants, during food processing, and also after human

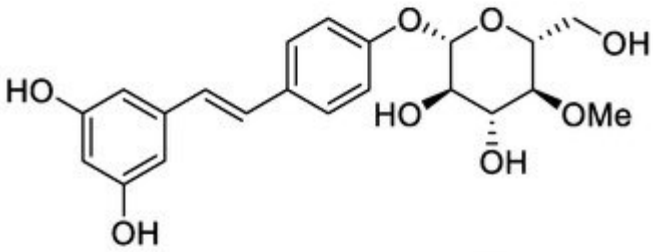
consumption, which can be protected by glycosylation [25]. The incorporation of sugar moieties into different types of pharmacophores, natural products, or prodrugs has been proven to improve anti-cancer activities [26].

Several glycosyl derivatives of resveratrol have been recognized in the roots of *Poligonum cuspidatum* such as piceid (3-*O*-*β*-D-glucosyl resveratrol), resveratroloside (4′-*O*-*β*-D-glucosylresveratrol), and 4′-*O*-*β*-D-glucosyl piceatannol [27]. Piceid has been shown to exhibit a broad range of biological activities [28].

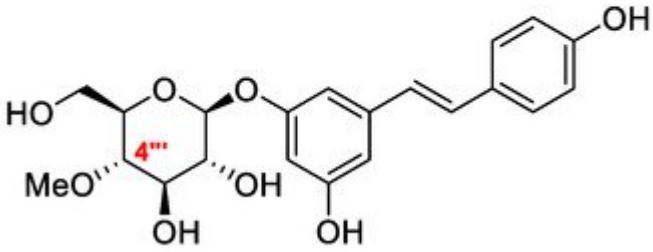
The fungus *Beauveria bassiana* is the most frequently used biocatalyst and has been used to transform more than 300 bioactive compounds [29][30]. For instance, *B. bassiana* ATCC 7159 has been used for the biotransformation of curvularin and kaempferol, leading to the production of new metabolites resulting from 4-*O*-methyl glucosylation of the substrate, and was highly selective among different hydroxyl groups in the same molecule [30]. Recently, resvebassianol A (**2**) shown in **Figure 1**, was identified through biotransformation of resveratrol by *B. bassiana* and exhibited important pharmacological activities such as inhibition of inflammatory cytokine expression and cell rejuvenation. Moreover, compared with resveratrol, resvebassianol A proved to be less toxic and more stable [31].



Resveratrol (1)



resvebassianol A (2)



3-*O*-Glycosylated resveratrol (3)

Figure 1. Chemical structures of resveratrol **1**, resvebassianol A (**2**), and 3-*O*-*β*-(4‴-*O*-methylglucopyranosyl) resveratrol **3**.

Several synthetic approaches for the formation of glycosidic bonds to phenolic OH in resveratrol have been reported. Direct coupling of resveratrol with a bromo-glucuronide donor was performed by Wang et al. for the synthesis of two glycoconjugates [32]. Coupling of resveratrol with glucuronyl bromide was performed using silver carbonate as an activator, in order to produce glucuronide-conjugated resveratrol in low yield, possibly due to the low solubility of resveratrol in organic solvents. Lucas et al. synthesized resveratrol 3-*O*-*β*-D-glucuronide by coupling a trichloroacetimidate glycosyl donor with protected resveratrol using TMSOTf and $BF_3.OEt_2$ as promoters [3]. Learmonth also synthesized two glucuronide conjugates of resveratrol, in which palladium-catalyzed Heck coupling of an iodo-*O*-*β*-D-glucuronate derivative and its corresponding styrene was adopted [33].

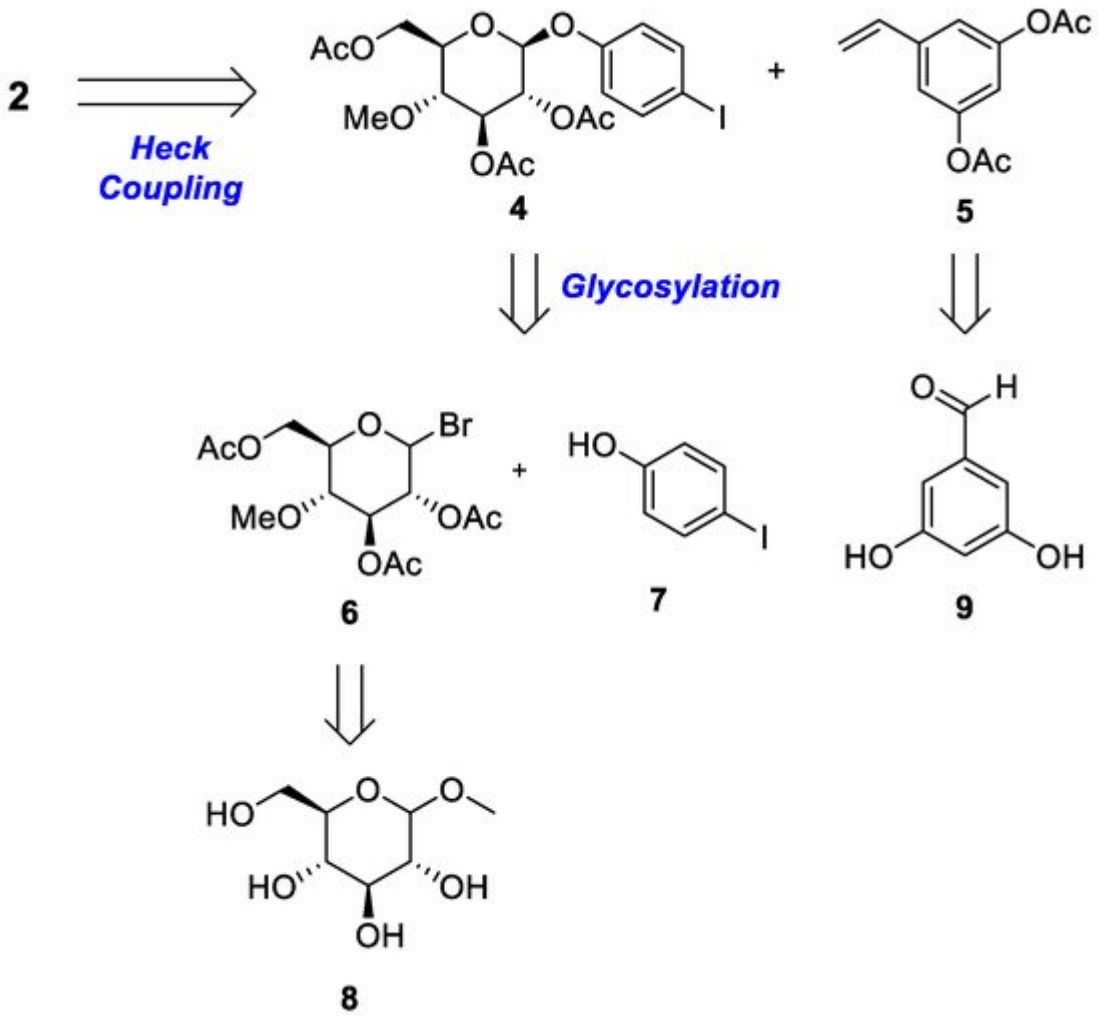
The structural uniqueness and natural resource scarcity of resvebassianol A for biological evaluation prompted us to develop an efficient synthetic method for the metabolite. In this study, we report the total synthesis of resvebassianol A (**2**), a metabolite of resveratrol by *B. bassiana*, and its regiomer, 3-*O*-*β*-(4‴-*O*-methylglucopyranosyl)resveratrol (**3**).

2. Results and Discussion

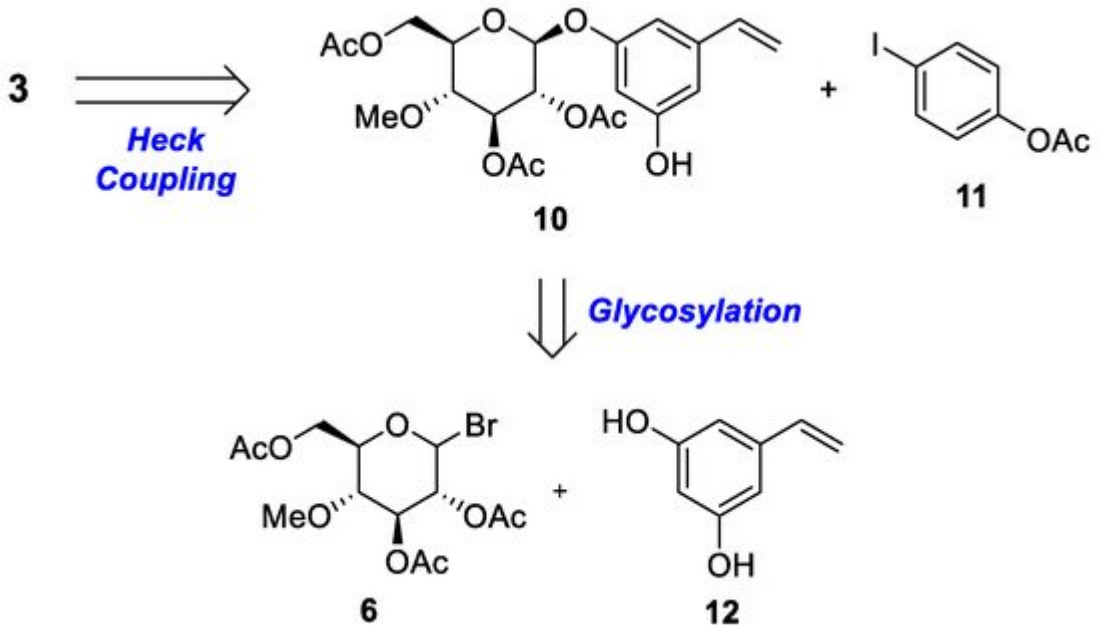
2.1. Retrosynthesis

Metabolite **2** and its regiomer **3** consist of a glycone attached to the aglycone moiety. The glycone moiety is 4-*O*-methyl glucopyranose, whereas the aglycone is a functional resveratrol featuring a stilbene core with a polyhydroxy group. Metabolite **2** is a structure in which 4-*O*-methylglucopyranose is attached to the 7-position hydroxyl group of resveratrol, whereas its regiomer **3** consists of a glycosyl moiety attached to the 3-position hydroxyl group of resveratrol. The synthesis of both metabolites involves a glycosylation reaction that introduces methylated glucose as the core reaction and the Heck reaction to form a stilbene skeleton [34].

Scheme 1 and Scheme 2 provide a retrosynthetic methodology for the synthesis of both metabolites **2** and **3**. Stilbene moiety **2** and its regiomer **3** were constructed via palladium-catalyzed Heck coupling. The rate-limiting step of the glycosylation reaction was performed with selectively protected compound **6** and commercially available iodophenol **7**. Compound **10** was obtained from the glycosylation of compound **6** and styrene **12**, which was synthesized from readily available dihydroxy benzaldehyde **9**.



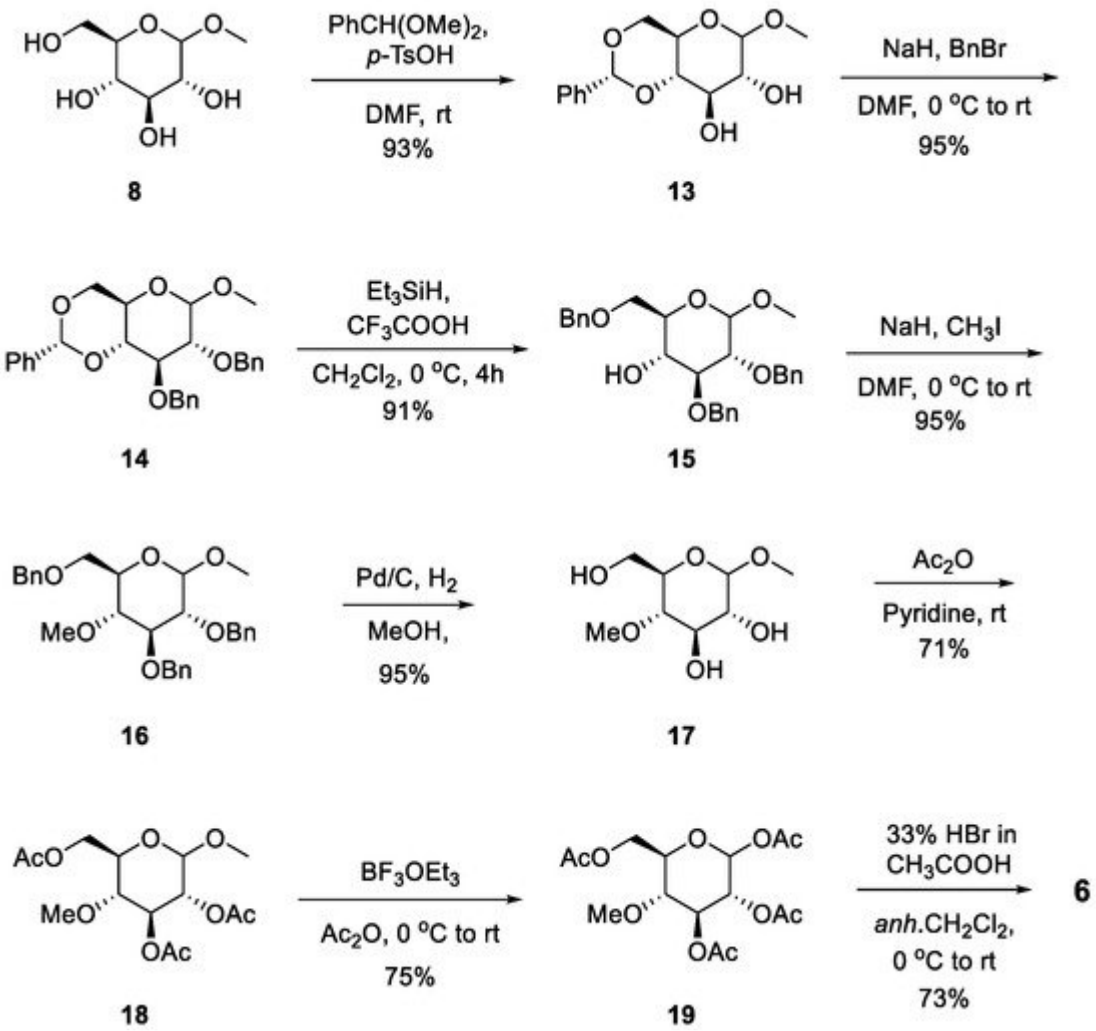
Scheme 1. Retrosynthetic analysis of 4′-*O*-*β*- (4‴-*O*-methylglucopyranosyl)resveratrol **2**.



Scheme 2. Retrosynthetic analysis of 3-*O*-*β*-(4‴-*O*-methylglucopyranosyl)resveratrol **3**.

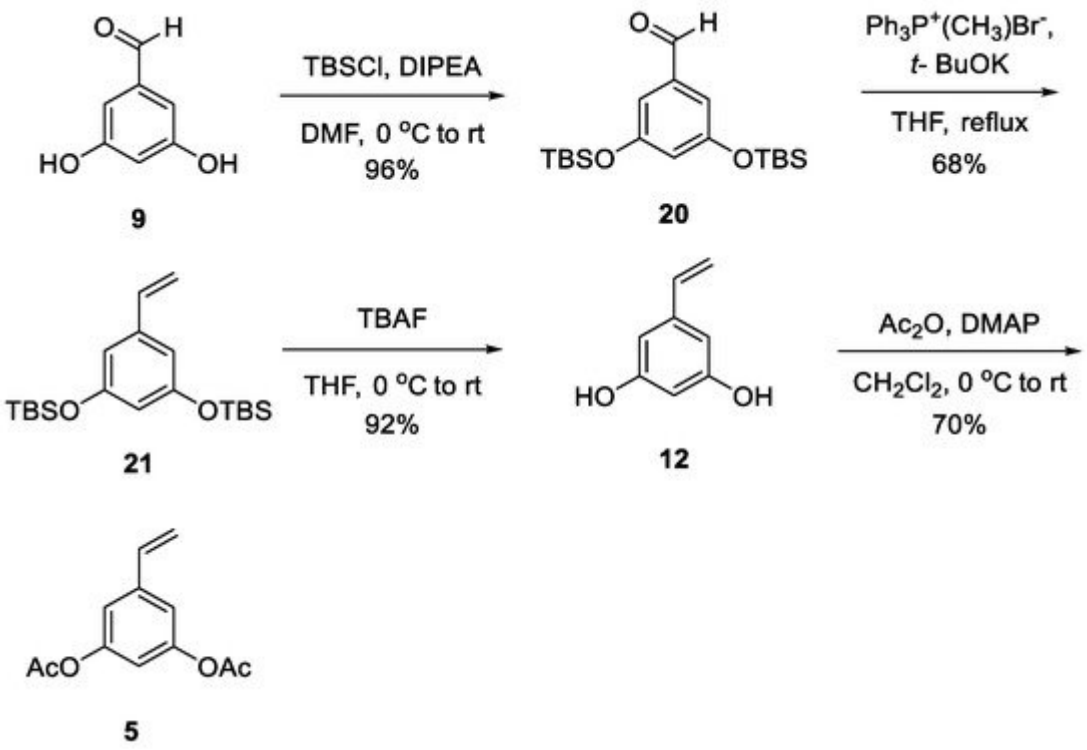
2.2. Chemistry

The reaction commenced with the preparation of the glycosyl donor, 4-O-methylglycopyranosyl bromide **6**, as shown in Scheme 3, which involves eight steps from commercially available methyl-α-D-glucopyranoside **8**. Regioselective protection of 4, 6-diol from the starting material was accomplished by the introduction of a 4,6-O-benzylidene group using benzaldehyde dimethyl acetal under acidic conditions, yielding protected compound **13**. 2,3-Di-O-benzylation of **13** generated **14** using NaH and BnBr. Further regioselective opening of the benzylidene ring of intermediate **14** was conducted with the help of triethylsilane (TES) and trifluoroacetic acid (TFA) to obtain alcohol **15** [35]. Methylation of compound **15** with NaH and MeI in N, N-dimethylformamide yielded product **16**, followed by hydrogenolysis to yield product **17**. Acetylation of the hydroxy groups of **17** was performed using pyridine and acetic anhydride to yield **18**, followed by the replacement of an anomeric methoxy group with an acetoxy group using boron trifluoride diethyl etherate to yield **19**. Finally, grafting of the anomeric acetoxy group was performed to incorporate bromine using HBr (33% in acetic acid) to yield acylated glycosyl bromide **6** at 73% [36]. As the final compound, 4-O-methylglucopyranosyl bromide **6**, has poor chemical stability, it is suitable to obtain a large amount of acetate compound **19** and synthesize **6** immediately when necessary.



Scheme 3. Synthesis of 4-O-methylglucopyransyl bromide **6**.

3,5. Dihydroxystyrene **12** and 3,5 diacetoxyystyrene **5** were synthesized from commercially available 3,5-dihydroxy benzaldehyde **9** according to Scheme 4. Protection of the hydroxy group of **9** with TBDMS yielded **20**, and the Wittig reaction yielded olefin **21** using methyltriphenylphosphonium bromide under basic conditions. The TBDMS group in intermediate **21** was removed using tetrabutylammonium fluoride (TBAF) to furnish dihydroxy styrene **12**, and further acetylation of both hydroxy groups resulted in **12** at 70% yield.

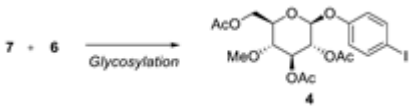


Scheme 4. Synthesis of diacetoxystyrene **5** and dihydroxystyrene **12**.

After obtaining **6** and **12**, the next target was to synthesize substrates **4** and **10**, which participate in the Heck reaction for the synthesis of the stilbene core.

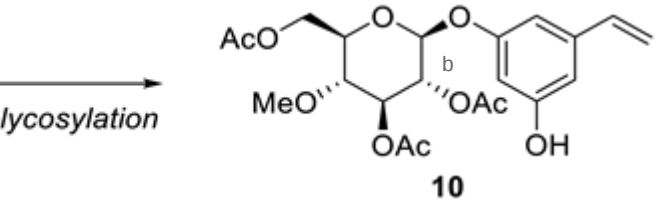
We performed glycosylation of both iodophenol **7** and dihydroxystyrene **12** separately with 4-*O*-methylglycopyranosyl bromide **6** under different reaction conditions, as shown in **Table 1** and **Table 2**. Using Ag_2CO_3 in acetonitrile produced low-yield glycoside products **4** and **10** up to 16% and 19%, respectively. We attempted to improve glycosylation using a phase transfer catalyst (TBAB) in a two-phase system (aqueous NaOH and K_2CO_3) in $CHCl_3$. Unfortunately, the reaction yielded trace amounts. The reaction was incomplete, and the substrate was recovered for reuse. Bromide compound **6** can be decomposed into glycal by an alkaline water phase and phenoxide anion [37]. Therefore, excess use of water in the reaction lowers the yield of the compound. After utilizing several conditions (**Table 1** and **Table 2**), the glycosylation reaction under the phase transfer catalyst $BnNBu_3Cl$ and K_2CO_3 as a base at room temperature yielded product **4** at 57% yield. The desired mono-glucosylated product **10** was obtained at 40% yield along with the undesired di-glucosylated product as a mixture, which was separated by column chromatography.

Table 1. Optimization of glycosylation reaction for synthesis of **4** [a].



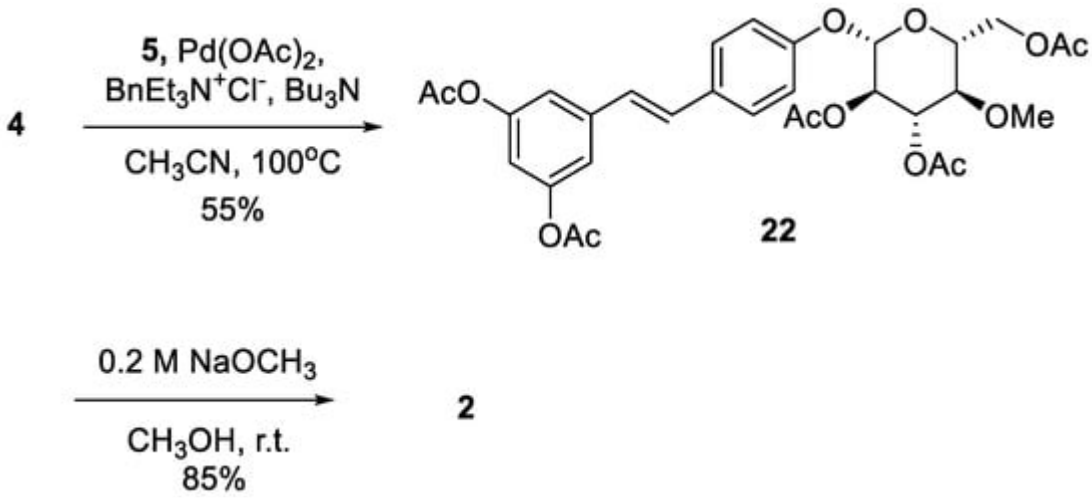
Entry [a]	Base	Reagent	Solvent	Temp. (°C)	Yield (%) [d]
1		Ag_2CO_3 (1eq)	CH_3CN	r.t	16
2 [b]	NaOH	TBAB	$CHCl_3$:H_2O (1:1)	45	14
3 [b]	K_2CO_3	TBAB	$CHCl_3$:H_2O (1:1)	45	17
4 [c]	K_2CO_3	$BnNBu_3Cl$	$CHCl_3$	r.t	57

[a] Reaction was carried out using 1 equiv of both starting materials **6** and **12**. [b] Base (1.1 eq), TBAB (0.2 eq), [c] Base (2.5 eq), and $BnNBu_3Cl$ (0.1 eq). [d] Isolated yield.

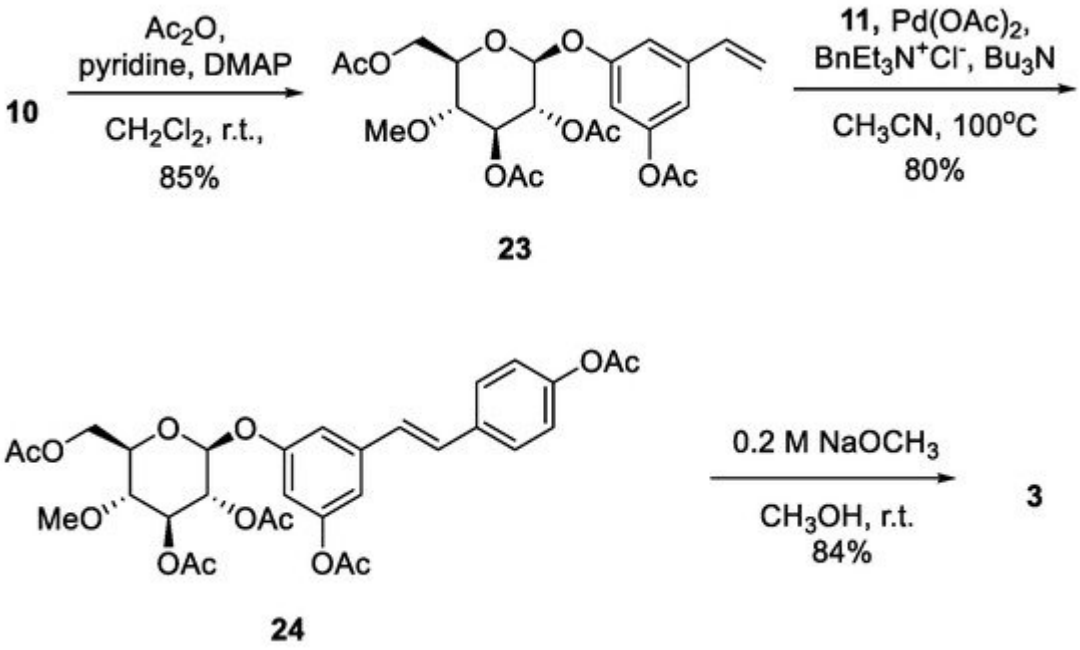


Entry	Base	Reagent	Solvent	Temp. (°C)	Yield (%) [d]
1		Ag_2CO_3	CH_3CN	r.t	19
2 [b]	NaOH	TBAB	$CHCl_3$:H_2O (1:1)	45	13
3 [b]	K_2CO_3	TBAB	$CHCl_3$:H_2O (1:1)	45	15
4 [c]	K_2CO_3	$BnNBu_3Cl$	$CHCl_3$	r.t	40

The hydroxy group of glycosylated product **10** was protected by acetylation to yield **23**. Similar to Scheme 5, the protected product **23** undergoes a palladium-catalyzed Heck reaction with 4-iodophenyl acetate **11** to build styrene compound **24** at 80% yield. Finally, basic hydrolysis of the acetyl protecting groups under a methanolic solution of sodium methoxide afforded another metabolite, **3**, at 84% yield (Scheme 6).



Scheme 5. Synthesis of 4′-*O*-*β*-(4‴-*O*-methylglucopyranosyl)resveratrol (**2**).



Scheme 6. Synthesis of 3-*O*-*β*-(4‴-*O*-methylglucopyranosyl)resveratrol (**3**).

3. Conclusions

In conclusion, an efficient total synthesis was performed for the preparation of resvebassianol A (**2**, a metabolite of resveratrol by *Beauveria bassiana*) and its regiomer (**3**) through glycosylation and a palladium-catalyzed Heck reaction. Resvebassianol A and regiomer **3** were synthesized in 11 and 12 linear steps, with overall yields of 7.5% and 6.3%, respectively. Incorporation of 4-*O* methyl glyosyl was performed through the glycosylation reaction and was optimized using a phase transfer catalyst with varying bases. This resulted in an elevated yield of up to 40% and 57%, respectively. Thus, this method can be helpful for the synthesis of metabolites that are difficult to obtain from plant sources and through microbial biotransformation. This strategy can also be used for the synthesis of other related metabolites.

References

1. Jang, M.; Cai, L.; Udeani, G.O.; Slowing, K.V.; Thomas, C.F.; Beecher, C.W.W.; Fong, H.H.S.; Farnsworth, N.R.; Kinghorn, A.D.; Mehta, R.G.; et al. Cancer Chemopreventive Activity of Resveratrol, a Natural Product Derived from Grapes. Science 1997, 275, 218–220.
2. Wang, Y.; Catana, F.; Yang, Y.; Roderick, R.; van Breemen, R.B. An LC-MS Method for Analyzing Total Resveratrol in Grape Juice, Cranberry Juice, and in Wine. J. Agric. Food Chem. 2002, 50, 431–435.
3. Lucas, R.; Alcantara, D.; Morales, J.C. A concise synthesis of glucuronide metabolites of urolithin-B, resveratrol, and hydroxytyrosol. Carbohydr. Res. 2009, 344, 1340–1346.

4. Fritzemeier, K.-H.; Kindl, H. Coordinate induction by UV light of stilbene synthase, phenylalanine ammonia-lyase and cinnamate 4-hydroxylase in leaves of vitaceae. Planta 1981, 151, 48–52.
5. Schultz, T.P.; Boldin, W.D.; Fisher, T.H.; Nicholas, D.D.; Mcmurtrey, K.D.; Pobanz, K. Structure-fungicidal properties of some 3- and 4-hydroxylated stilbenes and bibenzyl analogues. Phytochemistry 1992, 31, 3801–3806.
6. Takaoka, M. Of the phenolic substrate of hellebore (Veratrum grandiflorum Loes. fil.). J. Fac. Sci. Hokkaido Imper. Univ. 1940, 3, 1–16.
7. Quideau, S.; Deffieux, D.; Pouységu, L. Resveratrol Still Has Something To Say about Aging! Angew. Chem. Int. Ed. 2012, 51, 6824–6826.
8. Gülçin, I. Antioxidant properties of resveratrol: A structure—Activity insight. Innov. Food Sci. Emerg. Technol. 2010, 11, 210–218.
9. Vestergaard, M.; Ingmer, H. Antibacterial and antifungal properties of resveratrol. Int. J. Antimicrob. Agents 2019, 53, 716–723.
10. Lançon, A.; Frazzi, R.; Latruffe, N. Anti-Oxidant, Anti-Inflammatory and Anti-Angiogenic Properties of Resveratrol in Ocular Diseases. Molecules 2016, 21, 304.
11. Raval, A.P.; Lin, H.W.; Dave, K.R.; DeFazio, R.A.; Morte, D.; Kim, E.J.; Perez-Pinzon, M.A. Resveratrol and Ischemic Preconditioning in the Brain. Curr. Med. Chem. 2008, 15, 1545–1551.
12. Cho, S.; Namkoong, K.; Shin, M.; Park, J.; Yang, E.; Ihm, J.; Thu, V.T.; Kim, H.K.; Han, J. Cardiovascular Protective Effects and Clinical Applications of Resveratrol. J. Med. Food 2017, 20, 323–334.
13. Yang, X.; Xu, S.; Qian, Y.; Xiao, Q. Resveratrol regulates microglia M1/M2 polarization via PGC-1α in conditions of neuroinflammatory injury. Brain Behav. Immun. 2017, 64, 162–172.
14. Moussa, C.; Hebron, M.; Huang, X.; Ahn, J.; Rissman, R.A.; Aisen, P.S.; Turner, R.S. Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer’s disease. J. Neuroinflamm. 2017, 14, 1–10.
15. Li, Y.-R.; Li, S.; Lin, C.-C. Effect of resveratrol and pterostilbene on aging and longevity. BioFactors 2018, 44, 69–82.
16. Kapadia, G.J.; Azuine, M.A.; Tokuda, H.; Takasaki, M.; Mukainaka, T.; Konoshima, T.; Nishino, H. Chemopreventive effect of resveratrol, sesamol, sesame oil and sunflower oil in the Epstein-Barr virus early antigen activation assay and the mouse skin two-stage carcinogenesis. Pharmacol. Res. 2002, 45, 499–505.
17. Szkudelska, K.; Nogowski, L.; Szkudelski, T. Resveratrol, a naturally occurring diphenolic compound, affects lipogenesis, lipolysis and the antilipolytic action of insulin in isolated rat adipocytes. J. Steroid Biochem. Mol. Biol. 2009, 113, 17–24.

18. Dermani, F.K.; Saidijam, M.; Amini, R.; Mahdavinezhad, A.; Heydari, K.; Najafi, R. Resveratrol Inhibits Proliferation, Invasion, and Epithelial-Mesenchymal Transition by Increasing miR-200c Expression in HCT-116 Colorectal Cancer Cells. J. Cell. Biochem. 2017, 118, 1547–1555.
19. Porcu, M.; Chiarugi, A. The emerging therapeutic potential of sirtuin-interacting drugs: From cell death to lifespan extension. Trends Pharmacol. Sci. 2005, 26, 94–103.
20. Shakibaei, M.; Harikumar, K.B.; Aggarwal, B.B. Resveratrol addiction: To die or not to die. Mol. Nutr. Food Res. 2009, 53, 115–128.
21. Baur, J.A.; Sinclair, D.A. Therapeutic potential of resveratrol: The in vivo evidence. Nat. Rev. Drug Discov. 2006, 5, 493–506.
22. Walle, T. Bioavailability of resveratrol. Ann. N. Y. Acad. Sci. 2011, 1215, 9–15.
23. Shimoda, K.; Kubota, N.; Uesugi, D.; Hamada, H.; Tanigawa, M.; Hamada, H. Synthesis and pharmacological evaluation of glycosides of resveratrol, pterostilbene, and piceatannol. Ann. N. Y. Acad. Sci. 2015, 1348, 141–149.
24. Biasutto, L.; Marotta, E.; Bradaschia, A.; Fallica, M.; Mattarei, A.; Garbisa, S.; Zoratti, M.; Paradisi, C. Soluble polyphenols: Synthesis and bioavailability of 3,4′,5-tri(α-d-glucose-3-O-succinyl) resveratrol. Bioorganic Med. Chem. Lett. 2009, 19, 6721–6724.
25. Regev-Shoshani, G.; Shoseyov, O.; Bilkis, I.; Kerem, Z. Glycosylation of resveratrol protects it from enzymic oxidation. Biochem. J. 2003, 374, 157–163.
26. Dembitsky, V.M. Astonishing diversity of natural surfactants: 5. Biologically active glycosides of aromatic metabolites. Lipids 2005, 40, 869–900.
27. Torres, P.; Poveda, A.; Jimenez-Barbero, J.; Parra, J.L.; Comelles, F.; Ballesteros, A.O.; Plou, F.J. Enzymatic Synthesis of α-Glucosides of Resveratrol with Surfactant Activity. Adv. Synth. Catal. 2011, 353, 1077–1086.
28. Cichewicz, R.H.; Kouzi, S.A. Biotransformation of Resveratrol to Piceid byBacillus cereus. J. Nat. Prod. 1998, 61, 1313–1314.
29. Holland, H.L.; Morris, T.A.; Nava, P.J.; Zabic, M. A new paradigm for biohydroxylation by Beauveria bassiana ATCC 7159. Tetrahedron 1999, 55, 7441–7460.
30. Zhan, J.; Gunatilaka, A.A.L. Selective 4′-O-methylglycosylation of the pentahydroxy-flavonoid quercetin byBeauveria bassianaATCC 7159. Biocatal. Biotransform. 2006, 24, 396–399.
31. Ha, S.K.; Kang, M.C.; Lee, S.; Darlami, O.; Shin, D.; Choi, I.; Kim, K.H.; Kim, S.Y. Generation of Stilbene Glycoside with Promising Cell Rejuvenation Activity through Biotransformation by the Entomopathogenic Fungus Beauveria Bassiana. Biomedicines 2021, 9, 555.

32. Wang, L.-X.; Heredia, A.; Song, H.; Zhang, Z.; Yu, B.; Davis, C.; Redfield, R. Resveratrol glucuronides as the metabolites of resveratrol in humans: Characterization, synthesis, and anti-HIV activity. J. Pharm. Sci. 2004, 93, 2448–2457.
33. Learmonth, D.A. A Concise Synthesis of the 3-O-β-D- and 4‘-O-β-D-Glucuronide Conjugates of trans-Resveratrol. Bioconjugate Chem. 2003, 14, 262–267.
34. Botella, L.; Nájera, C. Synthesis of methylated resveratrol and analogues by Heck reactions in organic and aqueous solvents. Tetrahedron 2004, 60, 5563–5570.
35. DeNinno, M.P.; Etienne, J.B.; Duplantier, K.C. A method for the selective reduction of carbohydrate 4,6-O-benzylidene acetals. Tetrahedron Lett. 1995, 36, 669–672.
36. Montero, J.-L.; Winum, J.-Y.; Leydet, A.; Kamal, M.; Pavia, A.A.; Roque, J.-P. A convenient synthesis of peracetylated glycosyl halides using bismuth(III) halides as catalysts. Carbohydr. Res. 1997, 297, 175–180.
37. Hongu, M.; Saito, K.; Tsujihara, K. Solid-Liquid Phase Transfer Catalyzed Novel Glycosylation Reaction of Phenols. Synth. Commun. 1999, 29, 2775–2781.