An expeditious chemo-enzymatic synthesis of dihydronorcapsaicin β-D-glucopyranoside

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Abstract

A seven-step and scalable synthesis of dihydronorcapsaicin β-D-glucopyranoside, non-pungent and adrenal secretion enhancement activity (diet effect), was accomplished from vanillin. A simple and inexpensive glucosyl donor, acetobromoglucoose, was reacted with phenolic hydroxyl group of α-iodovanillin nitrile under basic conditions. Another key step was the lipase-catalyzed coupling reaction of primary amine, which was resulted from the reduction of the glucosylated product, with methyl nonanoate.

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1. Introduction

Capsaicin has biological activities such as adrenal catecholamine secretion, which enhances energy metabolism, and is expected for ‘diet effect’ [1]. Its strongly pungent taste, which has very well been known as a spice for a long time, is an obstacle for intake in a large quantity. Towards the development of non-pungent, but biologically active materials, Watanabe, Kobata and co-workers have extensively studied, and capsiate (an ester) was elaborated [2]. As another approach to non-pungent, water-soluble, and biodegradable derivative, Hamada and co-workers enzymatically glycosylated capsaicins, by applying cultured plant cell of Phytolaca americana [3]. For example, the glucoside 2 was obtained in 46% yield from 1 (dihydronorcapsaicin, a synthetic analog of natural form). They also prepared 2 by way of a Lewis-acid catalyzed glycosylation of 1 with a glycosyl fluoride in 78% yield of this particular step [4]. In both cases, the syntheses were completed by the glycosylation at the final stage on dihydronorcapsaicin 1 itself, which had been synthesized separately in a chemical manner. Herein we describe a chemo-enzymatic, short-step total synthesis of 2 from vanillin 3a, a synthetic flavor produced in large quantities, as shown in Scheme 1. In contrast to Hamada’s syntheses, the glycosylation locates in an earlier stage, and the glycosyl donor is a very simple acetobromoglucoose, which can be prepared from glucose in one-pot reaction. Another feature is chemo- and regioselective introduction of fatty acid on the primary amine, by an enzyme-catalyzed aminolysis of ester.

2. Experimental section

All melting points were uncorrected. IR spectra were measured as thin films for oils or KBr disks of solid on a JASCO FT/IR-410 spectrometer. 1H NMR and 13C NMR spectra were measured at 270 MHz on a JEOL JNM EX-270 or at 400 MHz on a JEOL JNM GX-400 spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a Jeol JMS-700 spectrometer. Optical rotation values were recorded on a Jasco DIP 360 polarimeter. Silica gel 60 (spherical, 100–210 μm, 37558-79) of Kanto Chemical Co. was used for column chromatography. Preparative TLC was performed with E. Merck silica gel 60 F254 plates (0.5 mm thickness, No. 5744).

2.1. 4-Hydroxy-3-iodo-5-methoxybenzaldehyde (3b)

A solution of vanillin 3a (2.5 g, 16.4 mmol) and iodine (4.99 g, 19.3 mmol) in aqueous ammonia (28% solution, 11 mL) and THF (16.4 mL) was stirred at room temperature for 1.5 h. The dark solution became colorless at the end of the reaction. The reaction was quenched with saturated Na2S2O3 solution. Then concentrated hydrochloric acid was added until...
the solution reached to pH 1. The reaction mixture was extracted with AcOEt three times. The combined organic layer was washed with 10% aqueous Na2S2O3, brine, dried over anhydrous Na2SO4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (22 g). Elution with hexane–AcOEt (2/1) afforded nitrile 4 (840 mg, 85%) as colorless solid; mp 131–133°C; IR (KBr, cm⁻¹) 3313, 3010, 2231, 1595, 1573, 1491, 1411, 1284, 1180, 1041, 856, 821, 615; ¹H NMR (270 MHz, CDCl₃) δ: 3.94 (s, 3H, OMe), 6.57 (s, 1H, OH), 7.06 (d, J = 1.6 Hz, 1H, H-6), 7.64 (d, J = 1.6 Hz, 1H, H-2); ¹³C NMR (100 MHz, CDCl₃) δ: 56.6, 80.9, 105.1, 113.4, 117.6, 135.2, 145.7, 150.0. Anal. calcd for C₅H₄NO₂: C 34.94, H 2.20, N 5.09; found: C 35.02, H 2.23, N, 5.05.

2.3. Vanillyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (6a)

Acetobromoglucose 5 (252.7 mg, 0.615 mmol) was added to a solution of 3a (926.4 mg, 6.09 mmol) and disopropylethylene (0.8 mL, 4.86 mmol) in CH₂CN (13.8 mL), and the mixture was stirred at room temperature for 21 h. Then the mixture was concentrated in vacuo and any trace of solvent and disopropylethylene were removed azeotropically with toluene twice. The residue was purified by silica gel column chromatography (39 g). Elution with hexane–AcOEt (1/1) gave the glycoside 6a (215.7 mg, 73%); mp 142–143°C (lit. [6] 144–145°C); [α]D° = -47.5° (c 1.00, CHCl₃); ¹H NMR (270 MHz, CDCl₃) δ: 2.04 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.08 (s, 3H, OAc), 3.85 (s, 3H, OMe), 4.19 (dd, J = 2.6, 12.6 Hz, 1H, H-6a), 4.28 (dd, J = 2.6, 12.6 Hz, 1H, H-6b). 7.26 (m, 3H, H-3, H-4), 5.35 (d, J = 7.4 Hz, 1H, H-1), 7.21 (d, J = 8.4 Hz, 1H, H-3'), 7.41 (dd, J = 1.8, 8.4 Hz, 1H, H-2'), 7.43 (d, J = 1.8 Hz, 1H, H-6'), 9.89 (s, 1H, CHO).

2.4. 4-Formyl-2-iodo-6-methoxyphenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (6b)

The glycoside 6b was obtained according to the procedure as above with acetobromoglucose 5 (13.3 mg, 0.32 μmol), aldehyde 3b (18.0 mg, 64.6 μmol), disopropylethylene (9.6 μL, 55.4 μmol) and in acetone (1 mL) for 20 h. The mixture was stirred further under reflux for 2 h. The workup and purification provide 6b (14.3 mg, 73%). ¹H NMR (270 MHz, CDCl₃) δ: 2.01 (s, 3H, OAc), 2.02 (s, 6H, OAc), 3.94 (s, 3H, OMe), 6.57 (s, 1H, OH), 7.26 (m, 3H, H-3, H-4), 5.35 (d, J = 7.4 Hz, 1H, H-1), 7.21 (d, J = 8.4 Hz, 1H, H-3'), 7.41 (dd, J = 1.8, 8.4 Hz, 1H, H-2'), 7.43 (d, J = 1.8 Hz, 1H, H-6'), 9.89 (s, 1H, CHO). Its structure was confirmed by the conversion to 6c, in a similar manner as described for the preparation of 4.

2.5. 4-Cyano-2-iodo-6-methoxyphenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (6c)

Acetobromoglucose 5 (205.8 mg, 0.50 mmol), nitrile 4 (172.8 mg, 0.628 mmol), and disopropylethylene (96 μL, 0.55 mmol) were reacted in CH₂CN (4.5 mL) under reflux for 2.5 h. The workup and purification provided 6c (220 mg,
71%); mp 174–175.5 °C; [α]D20 -8.7° (c 1.00, CHCl3); IR (KBr, cm−1) 2952, 2229, 1751, 1566, 1468, 1381, 1230, 1043, 862, 631; 1H NMR (400 MHz, DMSO-d6) δ: 1.95–2.05 (s, 12H, OAc), 3.85 (s, 3H, OMe), 3.96 (d, J = 1.9 Hz, 1H, H-6b), 4.04 (dd, J = 4.9, 11.2 Hz, 1H, H-6a), 4.09–4.10 (m, 1H, H-5), 4.95 (dd, J = 9.2, 9.3 Hz, 1H, H-4), 5.06 (dd, J = 7.8, 8.3 Hz, 1H, H-2), 5.38 (dd, J = 9.3, 9.7 Hz, 1H, H-3), 5.62 (d, J = 7.8 Hz, 1H, H-1), 7.61 (dd, J = 1.9 Hz, 1H, H-6'), 7.86 (d, J = 1.9 Hz, 1H, H-2'); 13C NMR (100 MHz, CDCl3) δ: 20.6, 20.7, 20.8, 20.9, 56.4, 61.7, 67.8, 71.8, 72.0, 72.7, 93.0, 99.8, 110.0, 115.7, 116.8, 135.0, 148.4, 151.2, 169.1, 169.2, 170.2, 170.3. Anal. calc'd for C22H24NO11I: C 43.65, H 4.00, N 2.31; found: C 43.60, H 4.00, N 2.29.

2.6. 4-Cyano-2-methoxyphenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (6d)

The iodoglycoside 6c (49.5 mg, 82 μmol) was added to a solution of 20% palladium on carbon (catalytic amount) in MeOH (4 mL)–H2O (1 mL) and K2HPO4 (21.7 mg, 0.125 mmol). The mixture was stirred under H2 of atmospheric pressure at room temperature for 23 h. The reaction mixture was filtered, and the residual solid was washed with MeOH. The combined filtrate was concentrated in vacuo and the solid was dissolved in MeOH and brine, dried over anhydrous Na2SO4 and concentrated in vacuo. The residue was purified by silica gel column chromatography (8 g). Elution with hexane–AcOEt (1/3) afforded 7a (34.0 mg, 66%) as solid and 7b (7.7 mg, 13%) as viscous oil.

**Compound 7a.** Mp 91–92.8 °C (from hexane–Et2O); [α]D20 −33.2° (c 0.49, EtOH); IR (KBr, cm−1): 3282, 2927, 2856, 1747, 1639, 1597, 1537, 1516, 1421, 1379, 1253, 1224, 1162, 1124, 1037, 985, 804, 773; 1H NMR (400 MHz, DMSO-d6) δ: 0.82 (t, J = 6.8 Hz, 3H, OMe), 1.0 (1H, br s), 1.50 (2H, br s), 1.95 (s, 3H, OAc), 2.00 (s, 9H, OAc), 2.10 (t, J = 7.3 Hz, 2H, CO2H), 3.70 (s, 3H, OMe), 4.02 (d, J = 11.7 Hz, 1H, H-6b), 4.12 (dd, J = 5.4, 11.7 Hz, 1H, H-6a), 4.25 (br s, 3H, ArCH2), 4.92–5.05 (m, 2H, H-2, H-4), 5.25 (d, J = 7.8 Hz, 1H, H-1), 5.35 (dd, J = 9.3, 9.8 Hz, 1H, H-3), 6.75 (d, J = 8.3 Hz, 1H, H-3'), 6.90 (s, 1H, H-6'), 7.00 (d, J = 8.3 Hz, 1H, H-2'), 8.25 (t, J = 5.6 Hz 1H, NH); 13C NMR (100 MHz, CDCl3) δ: 14.1, 20.7, 20.7, 20.7, 22.7, 25.8, 29.2, 29.3, 29.4, 31.8, 36.9, 43.3, 56.0, 61.9, 68.4, 71.2, 72.0, 72.6, 100.8, 112.3, 119.8, 120.2, 135.2, 145.3, 150.7, 169.2, 169.3, 170.1, 170.4, 172.8; FAB(+) mass% 622 (M+ − 1, 32), 292 (base peak), 155 (61); Anal. calc'd for C32H32NO11: C 75.90, H 7.27, N 2.25; found: C 75.90, H 7.24, N 2.22.

**Compound 7b.** [α]D20 −17.6° (c 0.56, EtOH); IR (cm−1): 3296, 2924, 2854, 1743, 1641, 1597, 1516, 1466, 1421, 1377, 1218, 1038, 982, 806, 723, 696, 615; 1H NMR (400 MHz, DMSO-d6) δ: 0.82 (t, J = 6.4 Hz, 6H, OMe), 1.13–1.33 (20H, br s), 1.50 (4H, br s), 1.95 (s, 3H, OAc), 1.98 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.10 (t, J = 7.6 Hz, 2H, CO2H), 2.19 (t, J = 7.1 Hz, 2H, CO2H), 3.70 (s, 3H, OMe), 4.12 (d, J = 11.2 Hz, 1H, H-6b), 4.15 (dd, J = 5.4, 11.2 Hz, 1H, H-6a), 4.20 (br s, 3H, ArCH2), 4.92–5.05 (m, 2H, H-2, H-4), 5.25 (d, J = 8.3 Hz, 1H, H-1), 5.35 (dd, J = 8.3 Hz, 9.8 Hz, 1H, H-3), 6.75 (d, J = 8.3 Hz, 1H, H-3'), 6.85 (t, J = 5.9 Hz, 1H, NH); 13C NMR (100 MHz, CDCl3) δ: 14.1, 14.1, 20.6, 20.7, 20.7, 22.7, 22.7, 24.8, 25.8, 29.2, 29.2, 29.2, 29.3, 29.3, 31.8, 31.8, 34.1, 36.8, 43.4, 56.0, 61.8, 68.4, 71.2, 72.0, 72.6, 100.8, 112.3, 120.2, 135.2, 145.3, 150.7, 169.2, 169.2, 170.1, 172.9, 173.3; FAB(+) mass% 720 (M+ − 1, 6), 418 (24), 292 (64), 157 (base peak).
A solution of 7a (13 mg, 0.021 mmol) and triethylamine (9.3 μL) in MeOH (0.4 mL) was stirred under reflux for 6 h. The residue was concentrated in vacuo and any trace of solvent and triethylamine were removed azeotropically with toluene twice to give 2 (10.0 mg, quant.), which was washed with ethyl acetate three times to give an analytical sample (7.9 mg, 83% recovery); mp 170–171 °C. [α]D 38 − 39.4° (c 0.51, EtOH); IR (KBr, cm⁻¹): 3566, 3379, 3286, 2923, 2856, 1649, 1540, 1513, 1468, 1427, 1265, 1155, 1130, 1076, 1020, 896, 800, 733; 1H NMR (400 MHz, CD₃OD) δ: 0.89 (t, J = 7.6 Hz, 2H, Me); 2.21 (t, J = 7.6 Hz, 2H, COCH₂); 3.29–3.51 (m, 4H), 3.68 (dd, J = 7.6 Hz, 1H, H-3), 7.11 (d, J = 7.8 Hz, 1H, H-2'). 13C NMR (100 MHz, CD₃OD) δ: 14.5, 23.7, 27.1, 27.1, 30.3, 30.4, 33.0, 37.1, 43.8, 56.6, 62.5, 71.3, 74.8, 77.8, 78.1, 102.8, 113.1, 118.0, 121.2, 134.9, 147.0, 150.7, 175.9. Upon heating in vacuo, the sample reached a constant weight, which suggests monohydrate, and this formula was supported by elemental analysis. Anal. calcd for C₂₃H₃₉NO₉: C 58.33, H 8.30, N 2.96; found: C 58.07, H 8.19, N 2.86. Its 1H NMR spectrum was identical with that reported previously [4].

Introduction of electron-withdrawing iodine atom was really effective to enhance the nucleophilicity of phenols under basic conditions. In the case of 3b, the glycosyl donor was converted to 6b in 75% yield with the use of 2 equiv. of phenol. Furthermore, the equivalence of acceptor 4 could be suppressed as low as 1.25, while the reaction proceeded smoothly in 71% yield. The product 6c could be isolated in pure state, only by the single recrystallization of the crude material.

Initial attempts for the reduction of cyano group into aminomethyl group as well as the cleavage of carbon–iodine bond, by catalytic hydrogenation resulted only in a very poor yield of the desired product. Probably due to the adsorption of the resulted amine on the hydrogenation catalyst, the deiodinated nitrile 6d was the major product with a moderate yield (62%). In contrast, the conditions with NaBH₄–NiCl₂ [10] in ethanol enabled both of the desired reactions as mentioned above. In the crude amine, however, three of the four acetyl protective groups were removed under basic conditions, as suggested by 1H NMR. Any attempts involving the replace of solvent by less nucleophilic, sterically hindered tertiary alcohol, or reaction at lower temperature, could not prevent such undesired deprotection.

This situation prompted us to choose the lipase-catalyzed amidation reaction, which had been demonstrated in the preparation of capsaicins from vanillylamine and fatty acid esters by Watanabe, Kobata and co-workers [11]. The crude amine was treated with methyl nonanoate in the presence of C. antarctica lipase B (Chirazyme L-2) to provide a mixture of acylated products. Finally, dihydroorcapsaicin β-d-glucoside (2) was obtained in 34% yield from nitrile 6c, after exhaustive deprotection of all O-acyl groups.

The rate of the lipase-catalyzed acylation was enhanced very much, by applying reduced pressure [12] to remove liberated methanol continuously from the reaction mixture. In the enzyme-catalyzed acylation reactions, a hydroxyl group in serine residue of lipase attacks the carbonyl group of esters to form an enzyme–substrate complex. Next, primary amine or hydroxy nucleophiles would react on this complex to form new amide or ester bonds. After acetylation of the crude reaction product, the major components were isolated by silica gel column chromatography. In addition to the desired N-acylated product 7a (M+ 622) in 66% yield, NO-bis-acylated product 7b (M+ 720, 13% yield) was obtained. The position of O-acylation in 7b is tentatively assigned to be 6-position, as the lipase-catalyzed acylation preferentially occurs on sterically less-hindered primary positions. Finally, 7a was deprotected to 2 in quantitative yield by treatment with triethylamine in MeOH [13].

4. Conclusion

Dihydroorcapsaicin glucoside, a non-pungent capsaicin derivative was chemo-enzymatically synthesized via an expeditious route (seven steps) from commercially available vanillin as the starting material.

3. Results and discussion

For the introduction of nitrogen atom on vanillin, an attempted conversion of formyl group of 3a into cyanogroup of 4 according to Fang’s procedure (NH₃ aq., I₂) [7], iodination at ortho-position of phenol predominated, and an inseparable mixture of 3b and 4 was obtained. It turned out that even with a far excess of the reagents, the complete conversion to 4 was not feasible, as the less-soluble iodovanillin 3b precipitated as ammonium salt under the reaction conditions. On the other hand, lower equivalence of the reagents made the reaction possible to obtain pure 3b (58%). The subsequent conversion to the desired nitrile 4 was successful (85%) by way of aldoximes [8]. In this reaction, the elimination of trace of oxygen was essential, because the reaction is catalyzed by hydrogen iodide. To prevent the oxidation of hydrogen iodide to iodine, prior to the reaction, the mixture of all of the components was evacuated while applying ultrasonication.

A simple and inexpensive glycosyl donor, acetobromoglucose (5) was used for the coupling with phenols, under basic conditions (i-Pr₂NEt/CH₂CN) [9]. We compared glycosyl acceptors, 1, 3a, 3b, and 4. In the case of 1, a phenol with electron donating substituents the reaction was quite slow even under the refluxing temperature of acetonitrile. The reaction between 5 and 3a proceeded to give the corresponding glycoside 6a in 73% yield (based on the glycosyl donor), however, far excess (10 equiv.) of acceptor (3a) was required for the completion, which is useless in a practical sense.
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