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Synthesis and biological activities of analogs of D-glucosyl-L-tyrosine, a humoral factor that stimulates transcription of the acyl-CoA binding protein in the pheromone gland of the Silkmoth, *Bombyx mori*

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Abstract—β-D-Glucosyl-*O*-L-tyrosine (**1**) is a humoral factor that stimulates transcription of the acyl-CoA binding protein (ACBP) in the pheromone gland of the Silkmoth, *Bombyx mori*. This paper describes stereoselective synthesis of five analogs that changed the sugar and/or amino acid part in **1** and their stimulatory activities on the ACBP transcription in the pheromone gland of *B. mori*. Among the analogs tested, β-D-galactosyl-*O*-L-tyrosine showed a 1/5 potency compared to the activity of **1**.

1. Introduction

Acyl-CoA binding protein (ACBP) is a highly conserved 10-kDa intracellular lipid-binding protein that specifically binds medium- and long-chain fatty acyl-CoA esters with high affinity and is structurally highly conserved from yeast to mammals as well as insects.[1] While ACBP can functionally act as an acyl-CoA carrier or as an acyl-CoA pool maker within the cell,[2,3] the regulatory mechanisms underlying ACBP gene expression are unclear.

In the Silkmoth, *Bombyx mori*, a distinct ACBP designated as pgACBP, is specifically expressed in the pheromone gland (PG) during pheromonogenesis.[4] In addition, during pheromonogenesis, the PG cells accumulate a large number of lipid droplets within the cytoplasm just before eclosion.[5] Because these lipid droplets contain triacylglycerols (TGs) composed of unsaturated C_{16} and C_{18} fatty acids with the pheromone (bombykol, (*E*,*Z*)-10,12-hexadecadien-1-ol) precursor, $\Delta_{10,12}$-hexadecadienoic acid as a major component, they play a role in storing the bombykol precursor and eventually releasing it for bombykol production after eclosion in response to the neurohormone termed pheromone-biosynthesis-activating neuropeptide (PBAN).[5–7] We applied RNAi methodologies and demonstrated that targeted disruption of the pgACBP gene prevents TG accumulation within the cytoplasmic lipid droplets and consequently the availability of the bombykol precursors.[8] These results indicate the in vivo biological relevance of pgACBP in the biosynthesis of the TGs that constitute the cytoplasmic lipid droplets.

By exploiting this unique sex pheromone production system in the moth PG, we have also discovered that transcription of pgACBP is triggered by a hemolymph based humoral factor and provided evidence that transcription of some ACBPs can be triggered by specific humoral factors. Following purification and structure elucidation by means of high-resolution electrospray-ionization mass spectrometry (HR-ESIMS) and NMR analyses, in conjunction with stereochemical analyses using acid hydrolysates, we have identified the humoral factor to be β-D-glucosyl-*O*-L-tyrosine (**1**)[†] (Fig. 1).[9]

Keywords: D-Glucosyl-*O*-L-tyrosine; Acyl-CoA binding protein; D-Galactosyl-L-tyrosine.
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[†] β-D-Glucosyl-*O*-L-tyrosine (**1**) has been shown to be a transient metabolite of some insects and it appears to be the major tyrosine-storage metabolite for the production of tanning diphenol substrates in lepidoptera.[10–12]

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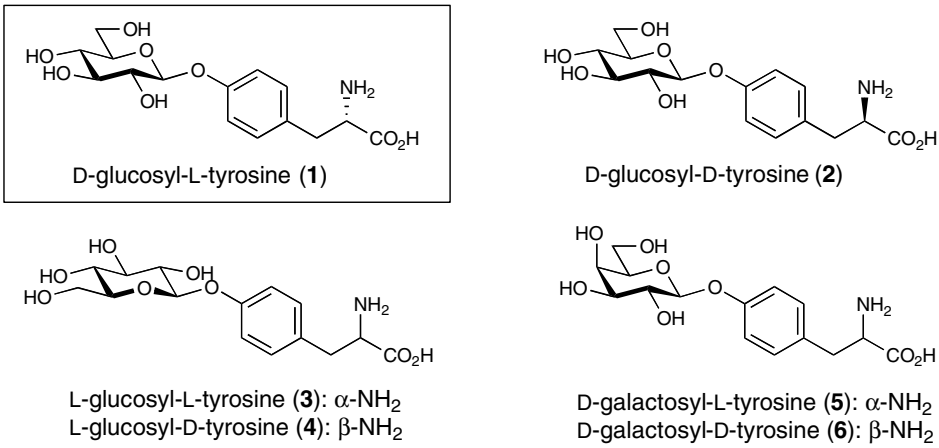


Figure 1. Structures of β-D-glucosyl-*O*-L-tyrosine (**1**) and its analogs.

Furthermore, by using an in vitro system with trimmed PG, we have demonstrated that synthetic **1** directly acts on the PG cells, although the molecular mechanisms underlying how the signal from **1** stimulates the transcription of pgACBP within the PG cells remain to be clarified.[9] As the first step in attempting to identify the mechanisms, here we describe the stereoselective syntheses of the diastereomers and galactosyl analogs **2**–**6** which could be used as ligand controls, and their stimulatory activities on the pgACBP transcription in the PG of *B. mori*.

2. Results and discussion

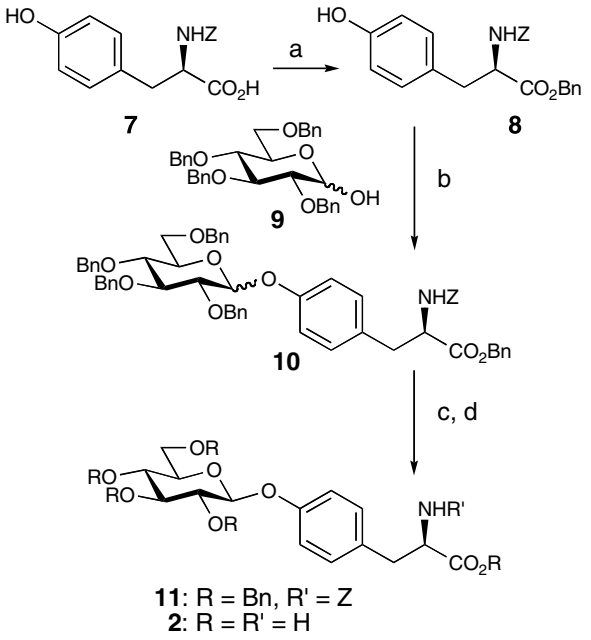
For preparation of analogs of **1**, formation of a 1,2-*trans* glycosidic bond between the tyrosine residue and the sugar part is a major problem.[13–15] Usually, 1,2-*trans* glycosidation is achieved by employing acylated sugars as a glycosyl donor. The use of acylated sugars, however, requires deprotection under alkaline conditions at the final stage, hiding the possibility that causes a partial epimerization of amino acid residue. Horvat et al.[13] reported that glycosidation of a benzylated D-glucosyl isourea[16] with amino acids afforded β-D-glucopyranosyl ethers stereoselectively. We planned to utilize Horvat's method[13] using a benzylated glycosyl isourea as a glycosyl donor. This strategy is expected to enable us to make analogs without technical problems arising from the handling of final products.

Synthesis began from the preparation of β-D-glucosyl-*O*-D-tyrosine (**2**). A commercially available *N*-benzyloxycarbonyl-D-tyrosine (**7**) reacted with benzyl bromide-sodium hydrogencarbonate to give *N*-benzyloxycarbonyl-D-tyrosine benzyl ester (**8**) in 94% yield (Scheme 1). This compound was treated with a benzylated glycosyl isourea, which was prepared in situ from 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (**9**) and *N*,*N*-dicyclohexylcarbodiimide (DCC) in the presence of copper chloride (CuCl) at 90 °C, giving glycosides **10** (73%; α-anomer/β-anomer = ca. 1/7 judged by ^{1}H NMR analysis) as a white solid. Although separation of the desired β-anomer **11** by chromatography on silica gel failed because compounds **10** showed a single spot on TLC, a fractional crystallization from ethanol afforded **11** as a pure form. In the ^{1}H NMR spectrum of **11**, the proton at C-1′ was observed at δ 4.82 as a doublet with $J_{1',2'} = 8.8$ Hz, showing the presence of a β-glycosidic bond. Finally, all benzyl protecting groups in **11** were removed by hydrogenolysis in the presence of 10% Pd/C in ethyl acetate/aq acetic acid to give analog **2**.

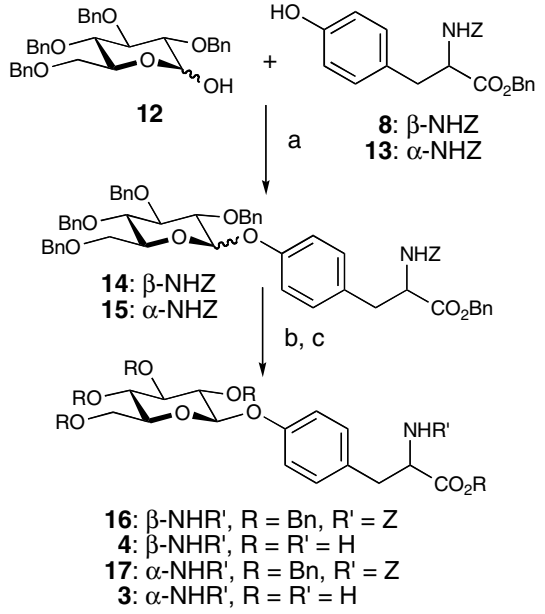
Synthesis of the L-glucose series was also conducted according to the procedure described above. Treatment of 2,3,4,6-tetra-*O*-benzyl-L-glucopyranose (**12**)[17] with DCC-CuCl followed by the addition of **8** afforded anomeric mixture **14** as a white solid in 40% yield (α/β = ca. 1/6) (Scheme 2). Different from the case of **10**, fractional crystallization failed. Consequently, the anomeric mixture **14** was employed in a reversed-phase HPLC to provide the desired isomer **16** as a pure form. Hydrogenation of **16** gave analog **4**. The use of the corresponding L-amino acid derivative **13**[18] instead of **8** gave the diastereomers **15** (α/β = 1/7) in 59% yield. Fractional crystallization followed by hydrogenation of the resulting compound **16** provided analog **3**.

Having completed the stereoselective synthesis of the diastereomers of β-D-glucosyl-*O*-L-tyrosine (**1**), we turned our attention to the synthesis of D-galactosyl analogs.[‡] There are no reports on this type of reaction of a galactose derivative. Therefore we initially searched the stereoselectivity of model compounds (Scheme 3). 2,3,4,6-Tetra-*O*-benzyl-D-galactopyranose (**18**) was treated with DCC and CuCl at 90 °C and then the resulting galactosyl isourea reacted with *p*-cresol (**19**) or methyl 4-hydroxyphenylacetate (**20**), providing the corresponding aryl glycosides **21** (47%) or **22** (51%), respectively. The 1,2-*trans*/*cis* selectivity in both cases was shown to be >4–5/1 by the ^{1}H NMR analyses. Encouraged by these results, we conducted a coupling reaction of **18** with **8** (Scheme 4). ^{1}H NMR spectra of **23** obtained in 67% yield revealed that this reaction also

[‡] In the surface layer glycoprotein of *Thermoanaeobacter thermohydrosulfuricus* strains, β-D-galactosyl-L-tyrosine (**5**) was found as the core component along with **1**.[19]

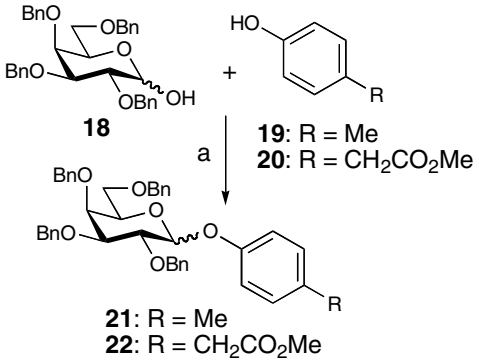


Scheme 1. Reagents and conditions: (a) BnBr, $NaHCO_3$, DMF, rt, 94%; (b) **9**, DCC, CuCl, 90 °C, and then **8**, 90 °C, 73%; (c) fractional crystallization from ethanol, 51%; (d) 10% Pd/C, H_2, ethyl acetate/aq acetic acid, rt, 75%.

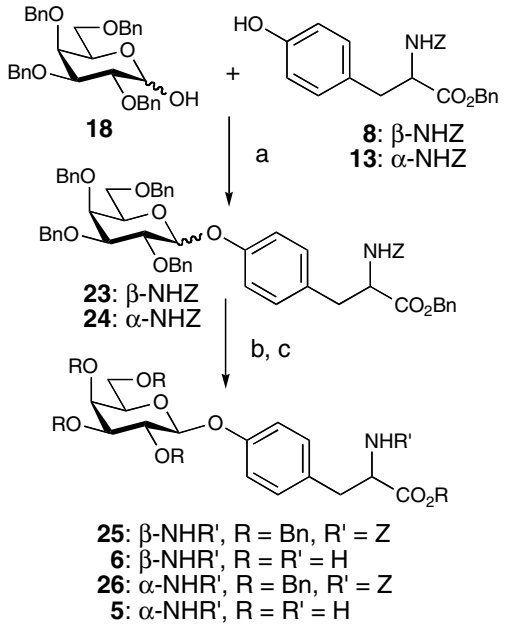


Scheme 2. Reagents and conditions: (a) **12**, DCC, CuCl, 90 °C, and then **8** or **13**, 90 °C, 40% for **14**, 59% for **15**; (b) fractional crystallization from ethanol, 74% for **16** or HPLC separation, 75% for **17**; (c) 10% Pd/C, H_2, ethyl acetate/aq acetic acid, rt, 89% for **4**, 59% for **3**.

proceeded in high β-selectivity (α/β = 1/8). Chromatography of **23** on silica gel followed by recrystallization from ethanol provided the β-anomer **25**, which underwent hydrogenolysis to give D-galacto analog **6**. Similarly, glycosidation between **18** and **13** afforded glycosides **24** (α/β = 1/5), from which β-glycoside **26** was separated by HPLC separation and recrystallization. Finally,



Scheme 3. Reagents and conditions: (a) **18**, DCC, CuCl, 90 °C, and then **19** or **20**, 90 °C, 47% for **21**, 51% for **22**.



Scheme 4. Reagents and conditions: (a) **18**, DCC, CuCl, 90 °C, and then **8** or **13**, 90 °C, 67% for **23**, 64% for **24**; (b) fractional crystallization from ethanol, 67% for **25** or HPLC separation, 55% for **26**; (c) 10% Pd/C, H_2, ethyl acetate/aq acetic acid, rt, 63% for **6**, 66% for **5**.

hydrogenation of **26** afforded the diastereomer **5**.[15] All analogs synthesized were submitted to the bioassay.

3. Biological activity[9] of synthetic analogs

To examine the biological activity that stimulates transcription of pgACBP, the synthetic analogs were injected into female P50 pupae three days before eclosion. For measurement of pgACBP transcript levels, RT-PCR analyses were performed by using total RNA prepared from the trimmed PGs 18 h after injection. We previously demonstrated that the β-D-glucosyl-*O*-L-tyrosine titer in the hemolymph of *B. mori* during pupal–adult molt reaches a maximum of as much as 5 mg/mL, the concentration of which is responsible for the up-regulation in pgACBP transcription in the PG during pheromonogen-

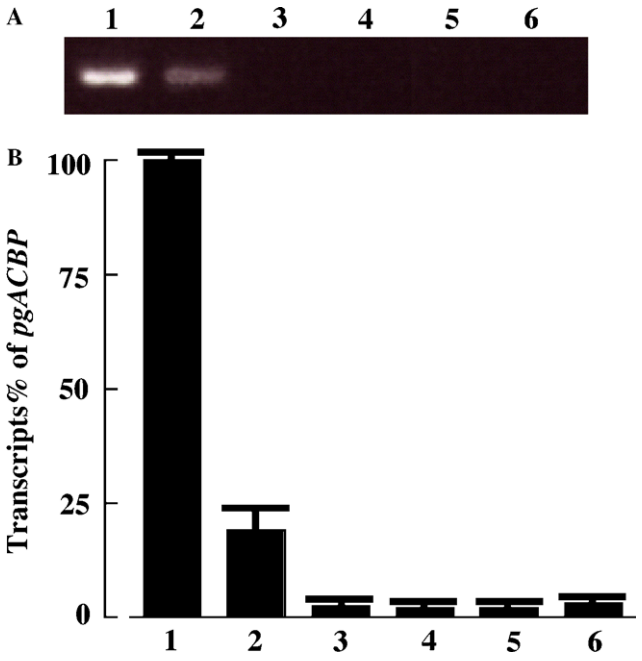


Figure 2. Stimulatory activities of β-D-glucosyl-*O*-L-tyrosine analogs on pgACBP transcription in the PG of *Bombyx mori*. (A) *pgACBP* transcript levels after injection of synthetic β-D-glucosyl-*O*-L-tyrosine analogs. Synthetic tyrosine glycosides (0.2 mg) were injected into day −3 female pupae. Trimmed PGs were prepared from pupae 18 h after injection and subjected to RT-PCR. Lane 1, β-D-glucosyl-*O*-L-tyrosine (**1**); lane 2, β-D-galactosyl-*O*-L-tyrosine (**5**); lane 3, β-D-galactosyl-*O*-D-tyrosine (**6**); lane 4, β-D-glucosyl-*O*-D-tyrosine (**2**); lane 5, β-L-glucosyl-*O*-L-tyrosine (**3**); lane 6, β-L-glucosyl-*O*-D-tyrosine (**4**). (B) The percentage of the *pgACBP* transcript level induced by D-glucosyl-L-tyrosine analogs injection. Results are expressed as percentage of control. Bars represent mean values ± SD ($n \geqslant 3$).

esis.[9] We, therefore, injected 0.2 mg of each synthetic analog into P50 pupae (final concentration, 5.0 mg/mL hemolymph). The results indicate that β-D-galactosyl-*O*-L-tyrosine (**5**) revealed the stimulatory activity albeit the potency was about 1/5 compared to that of **1** (Fig. 2; lanes 1–2), while the other synthetic analogs **2**–**4**, and **6** had no effect on the transcription of pgACBP (Fig. 2; lanes 3–6). These results show that both of the absolute configurations of the sugar part and the amino acid residue are important for stimulatory activity. Furthermore, the configurational change at the C-4 position in **1** influenced the activity to a lesser extent, suggesting that the possibility of artificial modifications around the position. This information would be useful for designing a bioprobe for understanding the mechanism of the transcription of pgACBP within the PG cells.

4. Experimental

4.1. General procedures

All reactions were carried out under an argon atmosphere, unless otherwise noted. Melting points were determined using a Yanaco MP-500 apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter. IR spectra were recorded with a JASCO VALOR-III spectrophotometer. ^{1}H NMR spectra were recorded at 400 or 600 MHz with JEOL α 400 or JNM-ECA 600 spectrometers, using tetramethylsilane as the internal standard. HPLC was performed with a Shiseido Capcell-pak C_{18} (20 × 250 mm) column in a Senshu Scientific Co. SSC-6300 F liquid chromatograph equipped with an SSC-5200 UV detector. Column chromatography was performed on Kanto silica gel 60 N (spherical, neutral; 40–100 μm). Merck precoated silica gel 60 F_{254} plates, 0.25 mm thickness, were used for analytical thin-layer chromatography. The solvent extracts were dried with magnesium sulfate, and the solutions were evaporated under diminished pressure at 40–42 °C.

4.2. *N*-Benzyloxycarbonyl-D-tyrosine benzyl ester (8)

To a vigorously stirred suspension of **7** (1.0 g, 3.2 mmol) and sodium hydrogencarbonate (530 mg, 6.3 mmol) in *N*,*N*-dimethylformamide (50 ml) was added dropwise benzyl bromide (1.9 ml, 15.9 mmol) at rt, and the mixture was stirred at rt for 21 h. After addition of ice-water, the resulting mixture was extracted with ethyl acetate. The extracts were washed with water, brine, dried, and concentrated. The residue was chromatographed on silica gel (*n*-hexane/ethyl acetate = 4:1) to give **8** (1.2 g, 94%) as a white solid.

Compound **8**; mp 119–120°C (lit.[18] 118–119 °C); $[\alpha]_D^{21}$ −5.7 (*c* 1.06, $CHCl_3$); $[\alpha]_D^{22}$ +13.8 (*c* 0.48, methanol); {for antipode: lit.[18] $[\alpha]_D$ −13.9 (methanol)}. IR (neat) 3312, 3032, 2953, 1720, 1692, 1514, 1250, 1213, 1174, 1055, 1028 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.23 (10H, m), 6.84 (2H, d, *J* = 8.8 Hz), 6.67 (2H, d, *J* = 8.8 Hz), 6.17 (1H, s), 5.24 (1H, brd, *J* = 8.3 Hz), 5.16, 5.11 (2H, each d, *J* = 12.2 Hz), 5.09, 5.07 (2H, each d, *J* = 12.2 Hz), 4.65 (1H, m), 3.07–3.01 (2H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.6, 155.8, 154.9, 136.0, 134.9, 130.4, 128.6, 128.5, 128.2, 127.1, 115.5, 67.3, 67.1, 54.9, 37.3. HRMS calcd for $C_{24}H_{24}NO_5$ $[M+H]^+$ 406.1654, found 406.1691.

4.3. 4-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-glucopyranosyl)-*N*-benzyloxycarbonyl-D-tyrosine benzylester (11)

To a stirred mixture of **9** (1.0 g, 1.85 mmol) and *N*,*N*-dicyclohexylcarbodiimide (384 mg, 1.86 mmol) was added copper chloride (32.0 mg, 0.32 mmol) and the mixture was stirred at 90 °C for 2 h. Then, **8** (500 mg, 1.23 mmol) was added and the resulting mixture was stirred at the same temperature for 1.5 h, cooled, diluted with dichloromethane, and then filtered through a pad of Celite. The filtrate was concentrated to give a syrup, which was chromatographed on silica gel (dichloromethane/ether = 30:1) to give **10** {830 mg, 73% (α/β = 1/7)} as a solid. Fractional crystallization from ethanol gave **11** (584 mg, 51%) as a white solid.

Compound **11**. Mp 104–105 °C; $[\alpha]_D^{21}$ −5.2 (*c* 1.03, $CHCl_3$). IR (neat) 3325, 1736, 1717, 1696, 1559, 1509, 1456, 1356, 1287, 1233, 1196, 1069 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36–7.17 (30H, m), 6.90 (4H, s), 5.22 (1H, d, *J* = 7.8 Hz), 5.16–4.49 (12H, m), 4.82 (1H, d, *J* = 8.8 Hz), 4.67 (1H, m), 3.79–3.57 (6H, m), 3.07

(2H, d, J = 5.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 156.5, 155.6, 138.4, 138.2, 138.1, 138.0, 136.1, 135.0, 130.4, 129.5, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 117.0, 101.6, 84.6, 81.9, 77.6, 75.7, 75.1, 75.0, 73.5, 68.7, 67.3, 67.0, 54.8, 37.3. HRMS calcd for $C_{58}H_{58}NO_{10}$ $[M+H]^+$ 928.4061, found 928.4076.

4.4. 4-*O*-(β-D-Glucopyranosyl)-D-tyrosine (2)

A mixture of **11** (103 mg, 0.11 mol) and 10% Pd/C (33 mg) in acetic acid/ethyl acetate/water (8:3:1; 3.2 ml) was vigorously stirred at rt under hydrogen for 12 h and then filtered through a pad of Celite. The filtrate was concentrated to give a syrup, which was treated with methanol to give **2** (29 mg, 75%) as a white powder.

Compound **2**. $[\alpha]_D^{22}$ −25.4 (c 0.11, H_2O). IR (neat) 3325, 1610, 1585, 1509, 1406, 1337, 1316, 1231, 1076, 1044, 988 cm^{-1}. ^{1}H NMR (600 MHz, D_2O): δ 7.28 (2H, d, J = 7.6 Hz), 7.12 (2H, d, J = 7.6 Hz), 5.12 (1H, d, J = 7.6 Hz), 3.94 (1H, br s), 3.92 (1H, dd, J = 12.6, 2.0 Hz), 3.74 (1H, dd, J = 12.6, 5.5 Hz), 3.62 (1H, m), 3.60 (1H, dd, J = 9.1, 8.6 Hz), 3.56 (1H, dd, J = 9.1, 7.6 Hz), 3.49 (1H, t, J = 9.1 Hz), 3.23 (1H, brd, J = 11.6 Hz), 3.08 (1H, m). ^{13}C NMR (100 MHz, D_2O): δ 176.9, 158.9, 133.7, 132.7, 120.0, 103.1, 79.1, 78.5, 75.9, 72.4, 63.5, 59.0, 38.5. HRMS calcd for $C_{15}H_{22}NO_8$ $[M+H]^+$ 344.1345, found 344.1364.

4.5. 4-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-L-glucopyranosyl)-*N*-benzyloxycarbonyl-D-tyrosine benzylester (16)

Treatment of **12** (835 mg, 1.54 mmol) and **8** (501 mg, 1.24 mmol) as described for preparation of **10** from **8** and **9** yielded **14** {456 mg, 40% (α/β = 1/6)}. A reversed-phase HPLC separation (acetonitrile/water = 10:1) of **14** (369 mg) gave **16** (273 mg, 74%) as a white solid.

Compound **16**. Mp 83–84 °C (methanol); $[\alpha]_D^{21}$ +3.1 (c 0.58, $CHCl_3$). IR (neat) IR 3350, 1717, 1541, 1522, 1456, 1235, 1206, 1070, 1059, 1013 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.17 (30H, m), 6.91 (4H, s), 5.24 (1H, d, J = 7.8 Hz), 5.13–4.49 (12H, m), 4.84 (1H, d, J = 8.3 Hz), 4.69 (1H, m), 3.79–3.67 (5H, m), 3.66–3.59 (1H, m), 3.07 (2H, d, J = 5.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.3, 156.5, 155.6, 138.4, 138.2, 138.0, 137.9, 136.2, 135.0, 130.4, 129.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 117.0, 101.7, 84.6, 81.9, 77.6, 75.7, 75.0, 74.9, 73.4, 68.7, 67.2, 67.0, 54.8, 37.3. HRMS calcd for $C_{58}H_{58}NO_{10}$ $[M+H]^+$ 928.4061, found 928.4080.

4.6. 4-*O*-(β-L-Glucopyranosyl)-D-tyrosine (4)

Treatment of **16** (100 mg, 0.11 mmol) as described for preparation of **2** yielded **4** (33 mg, 89%) as a white powder.

Compound **4**. $[\alpha]_D^{22}$ +70.0 (c 0.15, H_2O). IR (neat) 3350, 1635, 1558, 1508, 1458, 1240, 1190, 1072 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 7.27 (2H, d, J = 6.8 Hz), 7.11 (2H, d, J = 6.8 Hz), 5.12 (1H, d, J = 7.4 Hz), 3.94–3.88 (2H, brd), 3.74 (1H, dd, J = 12.2, 5.2, Hz), 3.64–3.57 (2H, m), 3.56 (1H, t, J = 8.8 Hz), 3.49 (1H, t, J = 8.7 Hz), 3.23 (1H, m), 3.08 (1H, m). ^{13}C NMR (100 MHz, D_2O): δ 174.8, 156.8, 131.6, 130.7, 117.8, 101.0, 76.9, 76.4, 73.8, 70.2, 61.4, 56.9, 36.5. HRMS calcd for $C_{15}H_{22}NO_8$ $[M+H]^+$ 344.1345, found 344.1306.

4.7. 4-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-L-glucopyranosyl)-*N*-benzyloxycarbonyl-L-tyrosine benzylester (17)

Treatment of **12** (814 mg, 1.51 mmol) and **13** (500 mg, 1.23 mmol) as described for preparation of **10** from **8** and **9** yielded **15** {578 mg, 59% (α/β = 1/7)}. Fractional crystallization of **15** (381 mg) from ethanol gave **17** (287 mg, 75%) as a white solid.

Compound **17**. Mp 104–105 °C; $[\alpha]_D^{27}$ +7.8 (c 0.92, $CHCl_3$). The ^{1}H and ^{13}C NMR data were in good agreement with those of **11**. HRMS calcd for $C_{58}H_{58}NO_{10}$ $[M+H]^+$ 928.4061, found 928.4050.

4.8. 4-*O*-(β-L-Glucopyranosyl)-L-tyrosine (3)

Treatment of **17** (100 mg, 0.11 mmol) as described for preparation of **2** yielded **3** (22 mg, 59%) as a white powder.

Compound **3**. $[\alpha]_D^{23}$ +27.0 (c 0.11, H_2O). The ^{1}H and ^{13}C NMR data were in good agreement with those of **2**. HRMS calcd for $C_{15}H_{22}NO_8$ $[M+H]^+$ 344.1345, found 344.1371.

4.9. *p*-Tolyl 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosides (21)

To a stirred mixture of **18** (158 mg, 0.29 mmol) and DCC (60 mg, 0,29 mmol) was added copper chloride (4.7 mg, 0.05 mmol) and the mixture was stirred at 90 °C for 2 h. Then, **19** (24 μl, 0.23 mmol) was added and the resulting mixture was stirred at the same temperature for 1.0 h, cooled, diluted with dichloromethane, and then filtered through a pad of Celite. The filtrate was concentrated and then chromatographed on silica gel (*n*-hexane/ethyl acetate = 30:1 → 20:1 → 10:1 → 4:1) to give **21** {71 mg, 47% (α/β = 1/5)} as a syrup.

Compound **21**. IR (neat) 3032, 2919, 1508, 1496, 1226, 1088 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) of anomeric mixture (α/β = ca. 1/1): δ 7.43–6.96 (24H, m), 5.47 (0.5H, d, J = 2.9 Hz, α-H_1), 5.03–4.35 (8H, m), 4.93 (0.5H, d, J = 7.8 Hz, β-H_1), 4.19 (0.5H, dd, J = 9.7, 2.9 Hz, α-H_2), 4.15 (0.5H, dd, J = 9.7, 2.4 Hz, α-H_3), 4.16–4.05 (1H, m, α-H_5, β-H_2), 4.06 (0.5H, d, J = 2.4 Hz, α-H_4), 3.94 (0.5H, d, J = 2.9 Hz, β-H_4), 3.69–3.54 (2.5H, m, α-H_6, β-H_3, H_5, H_6, $H_{6'}$), 3.49 (0.5H, dd, J = 9.3, 5.9 Hz, α − $H_{6'}$), 2.30 (3H, s). HRMS calcd for $C_{41}H_{43}O_6$ $[M+H]^+$ 631.3060, found 631.3070.

4.10. 4-(Methoxycarbonylmethyl)phenyl 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosides (22)

Treatment of **18** (173 mg, 0.32 mmol) and DCC (66 mg, 0.32 mmol) as described for preparation of **21** from **18** and **19** yielded **22** {92 mg, 51% (α/β = 1/4)} as a syrup.

Compound **22**. IR (neat) 3032, 2920, 1735, 1509, 1453, 1233, 1087 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) of anomeric mixture (α/β = 1/4): δ 7.44–7.17 (24H, m), 5.47 (0.2H, d, J = 3.4 Hz, α-H_1), 5.02–4.34 (8H, m), 4.96 (0.8H, d, J = 7.8 Hz, β-H_1), 4.19 (0.2H, dd, J = 10.2, 3.4 Hz, α-H_2), 4.15 (0.2H, dd, J = 10.2, 2.9 Hz, α-H_3), 4.14-4.05 (1H, m, α-H_5), 4.11 (0.4H, dd, J = 9.8, 7.8 Hz, β-H_2), 4.08 (0.2H, br s, α-H_4), 3.95 (0.8H, d, J = 2.9 Hz, β-H_4), 3.72–3.52 (1.6H, m, α-H_6, β-H_3, H_5, H_6, $H_{6'}$), 3.70 (0.6H, s), 3.69 (2.4H, s), 3.58 (2H, br s), 3.50 (0.2H, dd, J = 9.3, 5.9 Hz, α – $H_{6'}$). HRMS calcd for $C_{43}H_{45}O_8$ $[M+H]^+$ 689.3114, found 689.3102.

4.11. 4-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-*N*-benzyloxycarbonyl-D-tyrosine benzylester (25)

Treatment of **18** (503 mg, 0.93 mmol) and **8** (301 mg, 0.74 mmol) as described for preparation of **10** from **8** and **9** yielded **23** {405 mg, 67% (α/β = 1/8)}. Fractional crystallization of **23** (251 mg) from ethanol gave **25** (167 mg, 67%) a white solid.

Compound **25**. Mp 127–129 °C (ethanol); $[\alpha]_D^{26}$ −15.3 (*c* 0.61, $CHCl_3$). IR (neat) 3300, 1740, 1696, 1509, 1456, 1287, 1233, 1065 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.39–7.24 (30H, m), 6.89 (4H, s), 5.20 (1H, d, J = 8.3 Hz), 5.15–4.62 (10H, m), 4.91 (1H, d, J = 7.8 Hz), 4.65 (1H, m), 4.43, 4.38 (2H, each d, J = 11.7 Hz), 4.09 (1H, dd, J = 9.3, 8.3 Hz), 3.95 (1H, d, J = 2.4 Hz), 3.68–3.59 (4H, m), 3.05 (2H, d, J = 5.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.2, 156.5, 155.5, 138.4, 138.3, 138.2, 137.7, 136.1, 134.9, 130.2, 129.3, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.5, 127.4, 116.9, 101.8, 81.9, 79.0, 75.2, 74.4, 73.7, 73.4, 73.2, 72.9, 68.6, 67.1, 66.8, 54.8, 37.2. HRMS calcd for $C_{58}H_{58}NO_{10}$ $[M+H]^+$ 928.4061, found 928.4043.

4.12. 4-*O*-(β-D-Galactopyranosyl)-D-tyrosine (6)

Treatment of **25** (100 mg, 0.11 mmol) as described for preparation of **2** yielded **6** (24 mg, 63%) as a white powder.

Compound **6**. $[\alpha]_D^{25}$ −17.6 (*c* 0.10, H_2O). IR (neat) 3250, 1653, 1559, 1509, 1420, 1231, 1051 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 7.28 (2H, d, J = 8.3 Hz), 7.16 (2H, d, J = 8.3 Hz), 5.07 (1H, d, J = 7.3 Hz), 4.00 (1H, d, J = 2.9 Hz), 3.97 (1H, dd, J = 7.8, 5.4 Hz), 3.88 (1H, dd, J = 6.3, 5.9 Hz), 3.84–3.75 (4H, m), 3.25 (1H, dd, J = 14.6, 5.4 Hz), 3.10 (1H, dd, J = 14.6, 7.8 Hz). ^{13}C NMR (100 MHz, D_2O): δ 174.8, 156.9, 131.5, 130.3, 117.7, 101.4, 76.2, 73.3, 71.3, 69.2, 61.5, 56.8, 36.3; HRMS calcd for $C_{15}H_{22}NO_8$ $[M+H]^+$ 344.1345, found 344.1368.

4.13. 4-*O*-(2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl)-*N*-benzyloxycarbonyl-L-tyrosine benzylester (26)

Treatment of **18** (794 mg, 1.47 mmol) and **13** (476 mg, 1.17 mmol) as described for preparation of **10** from **8** and **9** yielded **24** {702 mg, 64% (α/β = 1/5)}.

A reversed-phase HPLC separation (acetonitrile/water = 10:1) of **24** (524 mg) gave **26** (288 mg, 55%) as a white solid.

Compound **26**. Mp 113–115 °C (ethanol); $[\alpha]_D^{25}$ −9.7 (*c* 0.96, $CHCl_3$). IR (neat) 3300, 1686, 1512, 1455, 1238, 1211, 1171, 1098, 1060, 1028 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.39–7.24 (30H, m), 6.88 (4H, d, J = 2.4 Hz), 5.19 (1H, d, J = 8.3 Hz), 5.16–4.62 (10H, m), 4.90 (1H, d, J = 7.8 Hz), 4.65 (1H, m), 4.43, 4.39 (2H, each d, J = 11.7 Hz), 4.09 (1H, dd, J = 9.8, 7.8 Hz), 3.95 (1H, d, J = 2.9 Hz), 3.68–3.59 (4H, m), 3.05 (2H, d, J = 5.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.4, 156.6, 155.6, 138.6, 138.5, 138.4, 137.8, 136.2, 135.1, 130.3, 129.4, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.6, 127.5, 117.2, 102.0, 82.1, 79.2, 75.4, 74.6, 73.8, 73.6, 73.4, 73.1, 68.8, 67.2, 67.0, 54.9, 37.4. HRMS calcd for $C_{58}H_{58}NO_{10}$ $[M+H]^+$ 928.4061, found 928.4033.

4.14. 4-*O*-(β-D-Galactopyranosyl)-L-tyrosine (6)

Treatment of **26** (101 mg, 0.11 mmol) as described for preparation of **2** yielded **5** (25 mg, 66%) as a white powder.

Compound **5**. $[\alpha]_D^{25}$ −78.9° (*c* 0.11, H_2O). IR (neat) 3250, 1600, 1717, 1509, 1474, 1458, 1399, 1233, 1049 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 7.29 (2H, d, J = 8.3 Hz), 7.14 (2H, d, J = 8.3 Hz), 5.07 (1H, d, J = 7.3 Hz), 4.02–3.96 (2H, m), 3.90–3.76 (4H, m), 3.26 (1H, dd, J = 14.6, 4.9 Hz), 3.10 (1H, dd, J = 14.6, 7.8 Hz). ^{13}C NMR (100 MHz, D_2O): δ 174.8, 156.9, 131.6, 130.4, 117.8, 101.5, 76.2, 73.4, 71.4, 69.3, 61.6, 56.8, 36.4. HRMS calcd for $C_{15}H_{22}NO_8$ $[M+H]^+$ 344.1345, found 344.1313.

4.15. Insects

Larvae of the inbred P50 strain of *B. mori*, a gift from T. Shimada (University of Tokyo, Japan), were reared on mulberry leaves and maintained under a 16L:8D photoperiod at 25 °C. Bioassays were performed using 5–10 female pupae 3 days before eclosion (day −3). Pupal age was determined based on the morphological characteristics as described.[7]

4.16. Bioassay in conjunction with RT-PCR

To examine the activity that stimulates transcription of pgACBP, test samples were diluted to 2 μL (50 μg/μL) with phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, and 1.5 mM KH_2PO_4 (pH 7.2)) and injected into 5–10 female pupae (day −3). After maintaining for 18 h at 25 °C, the injected pupae were dissected and their abdominal tips were excised into insect Ringer's solution (35 mM NaCl, 36 mM KCl, 12 mM $CaCl_2$, 16 mM $MgCl_2$, 274 mM glucose, and 5 mM Tris–HCl (pH 7.5)) for preparation of trimmed PGs.[20] For RT-PCR, total RNA was prepared from trimmed PGs by the method of Chomoczynski and Sacchi.[21] First-strand cDNA was synthesized from total RNA (500 ng) by using the RNA PCR kit (Takara Bio Inc., Japan) according to the manufacturer's instructions. Specific oligonucleotide primer pairs (for pgACBP, pgACBP-F1 [sense primer]: 5′-ATCAATATG TCTCTCCAAGAAAAA-3′ and pgACBP-R1 [anti-

sense primer]: 5′-CTTTTATTCTTTGAGGCCGATG-GA-3′) were designed based on their published sequences. PCR conditions consisted of 25 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. Following PCR, 5 μL aliquots were run on a 1.2% agarose gel in TAE buffer.

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