# Synthesis and Structure−Activity Relationship Study of Antidiabetic Penta-*O*-galloyl-D-glucopyranose and Its Analogues

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The rapid increase of obesity-associated diabetes has created urgent demands for more effective antidiabetic therapies and pharmaceuticals that are able to address the problems of hyperglycemia and weight gain simultaneously. Our previous studies indicated that the α- and *β*-anomers of penta-*O*-galloyl-D-glucopyranose (PGG), **2** and **3**, act as insulin mimetics that bind to and activate the insulin receptor, stimulate glucose transport in adipocytes, and reduce blood glucose and insulin levels in diabetic and obese animals. In addition, they inhibit differentiation of preadipocytes into adipocytes. These activities suggest that **2** and **3** may reduce blood glucose without increasing adiposity. To investigate the structure−activity relationship of **2** and **3**, four series of novel compounds were prepared and their glucose transport stimulatory activities were measured using a radioactive glucose uptake bioassay. The assay results indicate that both the glucose and the galloyl groups are critical to the activity of **2** and **3**. It appears that the glucose core provides an optimal scaffold to present the galloyl groups with the correct spatial orientation to induce activity. Moreover, the galloyl groups linked to the 1, 2, 3, and 4 positions of glucose are essential, while the galloyl group connected to the 6 position of **2** is unnecessary for the induction of activity. The discovery that two related novel compounds, 6-deoxytetra-*O*-galloyl-α-D-glucopyranose (**43**) and tetra-*O*-galloyl-α-D-xylopyranose (**59**), also possess glucose transport stimulatory activity suggests that **2** may be further modified around position 6 to modulate and enhance its efficacy. To test this hypothesis, we developed a new synthetic method that allows for the stereoselective preparation of derivatives of **2** that are modified on C-6. We found that 6-chloro-6-deoxy-1,2,3,4-tetra-*O*-galloyl-α-D-glucopyranose (**80**) exhibits a significantly higher glucose transport stimulatory activity than **2**. Its activity is comparable to that of insulin.

## Introduction

Diabetes mellitus, which is classified into type I diabetes (T1D) and type II diabetes (T2D), has become a serious health problem worldwide, particularly in developed countries.[1,2] Increasing attention has been focused on the elucidation of the disease mechanisms in order to develop new and more effective therapies.[3,4] T1D is an autoimmune disease characterized by a decrease in pancreatic secretion of insulin, while T2D is a result of insulin resistance and decreased insulin action.[1,5] The deficiency in insulin signaling in both types of diabetes leads to hyperglycemia.[1,5,6]

Visceral adiposity is associated with significantly increased risks for T2D,[7,8] and up to 90% of the T2D patients in the United States are either overweight or obese. Therefore, reducing adiposity, a major contributor to obesity, is beneficial for the prevention and treatment of T2D. Most current T2D drugs are both hypoglycemic and weight-gain-promoting, alleviating one problem (hyperglycemia) while aggravating another (weight gain).[4] Developing innovative antidiabetic therapeutics that simultaneously combat both hyperglycemia and overweight is highly desirable.[9−11]

Current treatments of diabetes are focused on reducing the blood glucose level with the help of insulin and other hypoglycemic agents. Mechanistic studies have shown that the transport of blood glucose into cells is largely induced and regulated by the circulating insulin through interaction with the insulin receptors (IR) located primarily in the plasma membrane of adipocytes and muscle cells.[12,13] IR is a heterotetrameric protein consisting of two α- and two *β*-subunits.[12,13] The two extracellular α-subunits form the insulin binding domain,[13,14] and the two *β*-subunits, covalently linked to the α-subunits and to each other by disulfide bonds, function as both the transmembrane and intracellular tyrosine kinase domains of IR.[12−14] The binding of insulin to the extracellular insulin binding site triggers a conformation change of the IR.[12−14] This induces the intracellular tyrosine kinase activity of the *β*-subunits, resulting sequentially in autophosphorylation of the tyrosine residues of the *β*-subunits, activation of intracellular protein factors PI-3K, Akt, and GLUT4, and finally transport of glucose into the target cells.[15] All protein factors involved in this insulin-mediated signaling pathway, including IR, can be potential pharmaceutical targets for diabetes treatment.[4,16]

IR has been a prime target for the development of antidiabetes pharmaceutics.[16−18] However, a high-resolution crystal structure of IR has not been established, and consequently the detailed information on insulin-IR binding is unavailable. Increased IR-mediated signaling is needed for the control of the blood glucose level in both types of diabetes.[19] Insulin has been the most frequently used antidiabetic agent that targets IR. However, insulin is a polypeptide that has to be invasively introduced (injected). Furthermore, it promotes adiposity and weight gain. Therefore, the discovery and development of nonpeptidyl, nonadiposity-promoting insulin supplements or replacements are highly desirable.[19−21]

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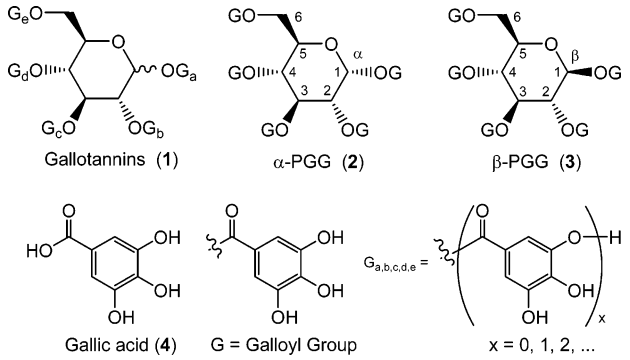



**Figure 1.** Structure of gallotannins and the two anomers of PGG, **2** and **3**.

In searching for new antidiabetic agents, we found that the extract of *Lagerstroemia speciosa* L. (also called Banaba) exhibits both glucose transport stimulatory activity and adipocyte differentiation−inhibitory activity in 3T3-L1 adipocytes.[22] Other groups also showed that the Banaba extract (BE) reduces blood glucose and adiposity of diabetic mice.[23,24] Our continued study led to the discovery that tannic acid (TA), a component of BE, exhibits the same activities.[25] This finding was consistent with a report showing that TA-related ellagitannins from BE exhibit a glucose transport stimulatory activity.[26] TA consists mainly of a group of structurally related polyphenolic compounds known as gallotannins (Figure 1) that possess a large variety of health-beneficial effects.[27−33] It is widely distributed in plant-based foods and in traditional medicinal herbs.[34−37]

Recently, we have identified penta-*O*-galloyl-*β*-D-glucopyranose (**3**), one of the major components of TA, as the most active compound in TA to induce the glucose transport stimulatory activity.[38] Two anomers of penta-*O*-galloyl-D-glucopyranose, **2** and **3**, exist in nature.[38] Both are composed of five gallic acid molecules (**4**) that are esterified with D-glucopyranose (Figure 1). Because of its potential health-beneficial bioactivities and abundant occurrence in nature,[39−43] **3** has been studied before. However, it was not previously known that **3** possesses anti-diabetic activity. We were the first to discover that both **2** and **3** exhibit glucose transport stimulatory and adipogenesis inhibitory activities in adipocytes.[38] The combination of the two biological activities suggests that **2** and **3** may be lead compounds for the development of novel antidiabetic therapeutics that are able to combat hyperglycemia and adiposity in T1D and/or T2D patients. Our signal pathway studies indicated that **2** binds to the α-subunit of the IR and induces IR autophosphorylation. It also activates PI-3K, Akt, GLUT4, protein factors and effectors in the cascade of IR-mediated signaling pathway, and the stimulation of glucose transport.[38] The results indicate that **2** is an insulin mimetic, and IR is the molecular target of **2** for its glucose transport stimulatory activity. However, a detailed study of the structural features of **2** and **3** required for activity have not been performed.

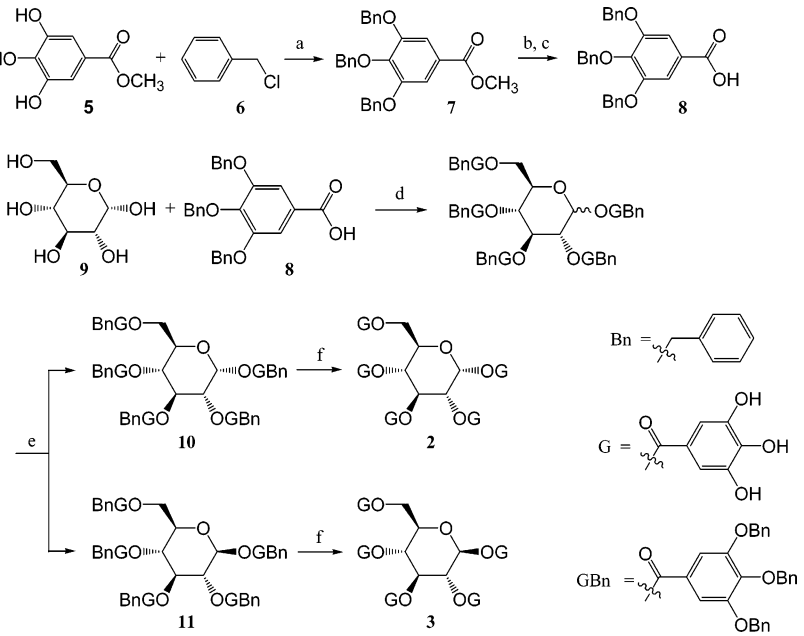
We hypothesized that the glucose core and the individual galloyl groups of **2** and **3** constitute important structural elements responsible for the induction of glucose transport into adipocytes. Several series of novel analogues of **2** and **3** were designed and synthesized to test this hypothesis. The glucose transport stimulatory activities of the new compounds were evaluated with a radioactive glucose uptake assay.[22,25,38]

## Chemical Synthesis

The key step in the known synthesis of **2** and **3** is the Steglich esterification of α-D-glucopyranose (**9**) with 3,4,5-tribenzyloxybenzoic (**8**) acid using dicyclohexylcarbodiimide (DCC) and *N*,*N*-(dimethylamino)pyridine (DMAP) as the coupling agents.[44,45] The procedure leads to epimerization at C-1 of the glucose ring. A mixture that contains approximately equal proportions of benzyl protected α- and *β*-anomers **10** and **11** forms (Scheme 1).

The procedure contains two steps that limit the scale of the synthesis to milligram quantities. A separation of the protected anomers **10** and **11** by column chromatography with pure dichloromethane as the eluent and a final purification of the individual deprotected products **2** and **3** by HPLC are needed to obtain pure products.[44] In our hands, the column chromatography with dichloromethane led to poor separations. We improved the process by adding toluene and ethyl acetate to the eluent. The best separation of the anomers was achieved

**Scheme 1.** Improved Synthesis of **2** and **3**[44,a]




[a] Reagents and conditions: (a) KI, $K_2CO_3$, acetone, reflux; (b) NaOH, ethanol, reflux; (c) HCl, water; (d) DCC, DMAP, $CH_2Cl_2$, 40 °C; (e) silica gel, dichloromethane/toluene/ethyl acetate (75:25:1); (f) $H_2$, THF, 10% Pd/C, 40 °C.

**Table 1.** Glucose Transport Stimulatory Activity of **2** and **3** and Their Analogues[a]

| compd | act.[c] | compd | act.[c] | compd | act.[c] | compd | act.[c] |
|---|---|---|---|---|---|---|---|
| pos[b] | 183 ± **8** | **22** | 20 ± 1 | **39** | 28 ± 1 | **61** | 21 ± 1 |
| neg[b] | 30 ± **1** | **24** | 16 ± 0 | **41** | 30 ± 1 | **63** | 23 ± 0 |
| **2** | 145 ± **5**[d] | **26** | 30 ± 0 | **43** | 115 ± **5**[d] | **65** | 14 ± 0 |
| **3** | 107 ± **9**[d] | **4** | 31 ± 3 | **45** | 15 ± 1 | **67** | 10 ± 0 |
| **9** | 34 ± 2 | **5** | 20 ± 0 | **47** | 22 ± 4 | **69** | 12 ± 1 |
| **12** | 31 ± 1 | **27** | 27 ± 1 | **49** | 19 ± 4 | **71** | 21 ± 1 |
| **13** | 24 ± 1 | **29** | 5 ± 1 | **51** | 23 ± 1 | **73** | 7 ± 0 |
| **14** | 34 ± 3 | **31** | 29 ± 2 | **53** | 34 ± 4 | **77** | 143 ± **10**[d] |
| **16** | 30 ± 1 | **33** | 15 ± 1 | **55** | 20 ± 0 | **80** | 181 ± **9**[d] |
| **18** | 27 ± 0 | **35** | 11 ± 0 | **57** | 19 ± 2 | | |
| **20** | 10 ± 0 | **37** | 7 ± 1 | **59** | 124 ± **11**[d] | | |

[a] All benzyl protected analogues failed to induce glucose uptake. The results are not shown. [b] In the glucose uptake assay, insulin-treated samples served as positive control (pos) and mock-treated (no compound) cell samples served as negative control (neg). [c] act. = activity for [1-$^3$H]-2-deoxy-D-glucose uptake, in Bq ± SE (SE = standard error). [d] $P < 0.001$ compared to negative control using unpaired *t*-test.

with a solvent mixture consisting of dichloromethane, toluene, and ethyl acetate in a ratio 75:25:1 (v/v/v).

The hydrogenolysis of **10** and **11** led to **2** and **3**, respectively. Our second improvement of the reported procedure consisted of the purification of the final product by crystallization. The α-isomer could be crystallized from water. A final purification by HPLC described in the original procedure[44] was unnecessary.

A third enhancement of the known synthesis was achieved by modifying the preparation of the benzyl protected gallic acid (**8**) utilized for the coupling reaction. The new procedure leads to a yield of 94% of 3,4,5-tribenzylgallic acid (**8**) starting from the commercially available methyl ester of gallic acid (**5**) (Scheme 1).

The improved chromatographic procedure was applicable in the synthesis of all derivatives of **2** and **3** shown in Charts 1−4. The crystallization of the final products succeeded for most α-isomers shown in Table 1. Only a few derivatives had to be purified by semipreparative HPLC (see Experimental Section).

The peripheral position of the galloyl groups in **2** and **3** led us to speculate about their significance for the interaction with the insulin receptor and the subsequent stimulation of glucose transport. To test the functional importance, we investigated a series of compounds that contain the glucose core without galloyl groups or with a reduced number of phenolic hydroxyl groups (Chart 1). Compounds **4** and **12**−**14** were commercially available, while compounds **15**−**26** were synthesized by esterification of glucose with a variety of protected phenolic acids using the method shown in Scheme 1.

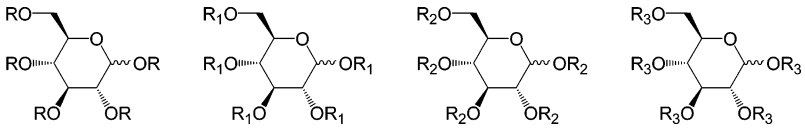
To investigate the importance of the glucose core for the bioactivity of **2** and **3**, we tested several commercially available gallic acid derivatives without a glucose core (Chart 2).

The functional importance of each individual galloyl group in **2** and **3** was tested in a third series of compounds consisting of monodeoxy-D-glucopyranose derivatives (Chart 3). Again, the procedure used for the synthesis of anomers **2** and **3** (Scheme 1) could be applied to prepare all compounds shown.

A final series of compounds were synthesized to test the dependence of activity on the spatial orientations of the galloyl groups in **2** and **3** and its analogues. Hexo- and pentopyranoses with one stereocenter inverted relative to the D-glucopyranose ring in **2** and **3** were prepared (Chart 4) from commercially available carbohydrates. In summary, 66 protected and unprotected analogues of **2** and **3** were synthesized with the help of our improved synthetic procedure. The only compounds previously known were **2**, **3**, **10**, **11**[44] and **25**, **26**.[46]

To confirm our preliminary results that suggested that derivatives of the α-anomer of glucopyranose retain activity if the structure around C-6 of **2** is changed, we developed a new synthesis for a derivative that contains all galloyl groups except for the one at C-6. The procedure is shown in Scheme 2. For the esterification, the acid chloride of protected gallic acid **74** was found to lead to the best yield. **74** was easily prepared from acid **8** by reaction with oxalyl chloride in the presence of catalytic amounts of dimethylformamide.

**Chart 1.** Analogues of **2** and **3** with Modified Galloyl Groups



| # | α/β | R | # | α/β | $R_1$ | # | α/β | $R_2$ | # | α/β | $R_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **9** | α | H | **15** | α | $R_1$Bn | **19** | α | $R_2$Bn | **23** | α | $R_3$Bn |
| **12** | α | Ac | **16** | α | $R_1$ | **20** | α | $R_2$ | **24** | α | $R_3$ |
| **13** | β | Ac | **17** | β | $R_1$Bn | **21** | β | $R_2$Bn | **25** | β | $R_3$Bn |
| **14** | α | Bz | **18** | β | $R_1$ | **22** | β | $R_2$ | **26** | β | $R_3$ |

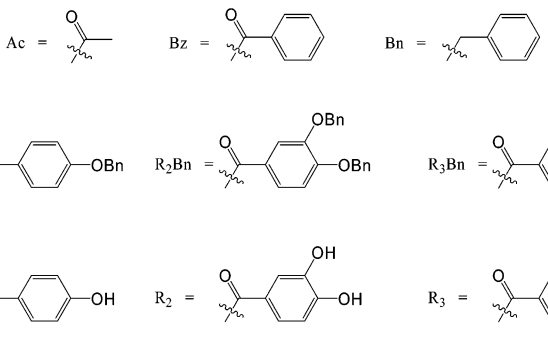
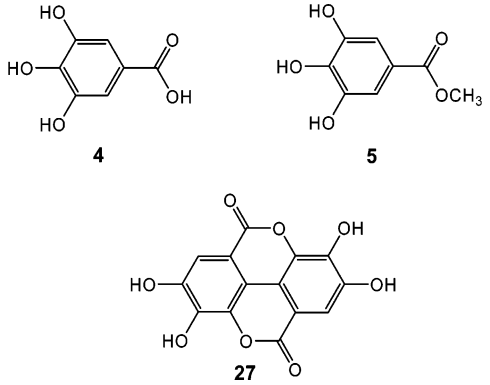


**Chart 2.** Structure of Commercially Available Gallic Acid Derivatives




The primary hydroxyl group at C-6 of glucopyranose was selectively protected with trityl chloride (TrCl).[47] Without isolating the product, all remaining hydroxyl groups were esterified with acid chloride **74**. The two-step procedure led to the epimerization of the anomeric center of the carbohydrate. The trityl protecting group of the mixture of α- and *β*-isomers **75** was removed with hydrobromic acid, and the anomers were separated by column chromatography. The benzyl protecting groups of the α-isomer were removed by hydrogenolysis, and the product was purified by crystallization from water.

We were interested in developing a synthesis that allows for an easy preparation of multigram quantities of derivatives of **2** needed for animal studies. Since commercially available glucose consists mostly of crystalline α-D-glucopyranose **9**, our goal was to find conditions that prevent or minimize the isomerization (mutarotation) of the α-configuration of the starting material to its *β*-form before the esterification can take place. We were successful by using the protected galloyl chloride **74** for the coupling reaction (Scheme 3). It was found that the selection of the solvent was crucial for the retention of the α-configuration during the transformation. Among the solvents tested, acetonitrile led to the best results. An α/*β* ratio of 96:4 could be obtained. After deprotection, the α-anomer could be further enriched to a purity >99% by crystallization from water. The new procedure does not need any chromatographic separation step. Gram quantities of **2** are easily synthesized.

The new synthetic approach was further tested by using the easily accessible 6-chloro-6-deoxy-α-D-glucopyranose **78**[48] as the carbohydrate starting material (Scheme 3). The α-selectivity of the transformation with acid chloride **74** was even higher than with **9**. Only α-anomer **79** could be detected by NMR spectroscopy of the product of the esterification. Final deprotection and crystallization from water afforded pure 6-chloro-6-deoxy-1,2,3,4-tetra-*O*-galloyl-α-D-glucopyranose (**80**).

## Results and Discussion

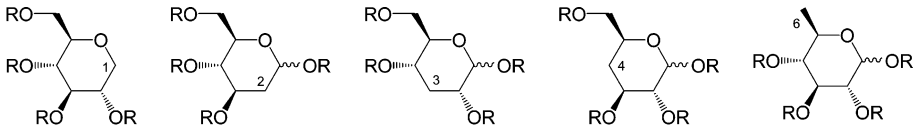
After modification of the known chemical synthesis as described in the chemistry section of this article (Scheme 1), the improved procedure was found to allow for the efficient preparation of **2** and **3** and its analogues. We successfully synthesized all our target compounds as shown in Charts 1, 3, and 4 using the same method with minor modifications. The glucose transport stimulatory activities of the target compounds in 3T3-L1 adipocytes were screened with an established glucose uptake bioassay that has been used extensively in diabetes studies[49,50] and previous research into **2** and **3**.[22,25,38]

Since **2** and **3** consist of D-glucopyranose connected to five gallic acid molecules (Figure 1), we assumed that both chemical components might be important for activity. In addition, the stereochemical arrangement of the galloyl groups was expected to have an influence as well. Evaluation of the target compounds confirmed previous results[38] that compounds **2** and **3** stimulate glucose transport in adipocytes in an insulin-like fashion (Table 1). The α-anomer **2** was found to be more active than *β*-anomer **3**. This key observation indicates that subtle structural differences have a significant impact on the glucose transport stimulatory activity of our lead compounds. In contrast, both protected precursor compounds **10** and **11** are inactive (Table 1), which suggests that unprotected hydroxyl functions on the galloyl groups are critical for activity.

Glucose (**9**) itself was found to be inactive. Commercially available acetates (**12** and **13**) and benzoates (**14**) of glucose were also inactive (Chart 1, Table 1). These results further emphasize the importance of the galloyl groups. Compounds **15**−**26** are esters of glucose and phenolic acids with only one or two hydroxyl groups. The observation that all compounds were inactive in our assay suggests that all three phenolic hydroxyl groups are required for activity.

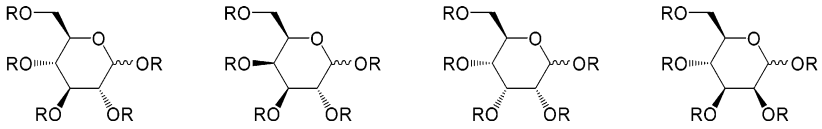
Our bioassay results reveal that gallic acid (**4**), methyl galloate (**5**), and ellagic acid (**27**) are inactive (Table 1). These commercially available compounds contain galloyl groups without a glucose core. The fact that all three compounds are inactive suggests that the glucose core is required as a scaffold to present one or more galloyl groups in a certain spatial arrangement to interact with and induce the receptor target.

**Chart 3.** Analogues of **2** and **3** with Four Galloyl Groups

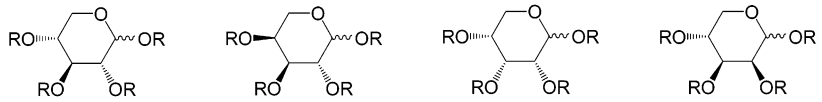



| 1-deoxy | | 2-deoxy | | | 3-deoxy | | | 4-deoxy | | | 6-deoxy | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| # | R | # | α/β | R | # | α/β | R | # | α/β | R | # | α/β | R |
| **28** | GBn | **30** | α | GBn | **34** | α | GBn | **38** | α | GBn | **42** | α | GBn |
| **29** | G | **31** | α | G | **35** | α | G | **39** | α | G | **43** | α | G |
| | | **32** | β | GBn | **36** | β | GBn | **40** | β | GBn | **44** | β | GBn |
| | | **33** | β | G | **37** | β | G | **41** | β | G | **45** | β | G |

**Chart 4.** Analogues of **2** and **3** with Modified Stereochemistry of the Carbohydrate Core

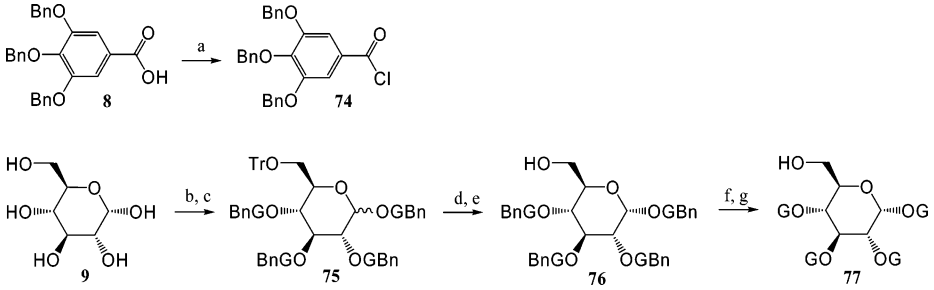


| D-Glucose | | | D-Galactose | | | D-Allose | | | D-Mannose | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| # | α/β | R | # | α/β | R | # | α/β | R | # | α/β | R |
| **10** | α | GBn | **46** | α | GBn | **50** | α | GBn | **54** | α | GBn |
| **2** | α | G | **47** | α | G | **51** | α | G | **55** | α | G |
| **11** | β | GBn | **48** | β | GBn | **52** | β | GBn | **56** | β | GBn |
| **3** | β | G | **49** | β | G | **53** | β | G | **57** | β | G |



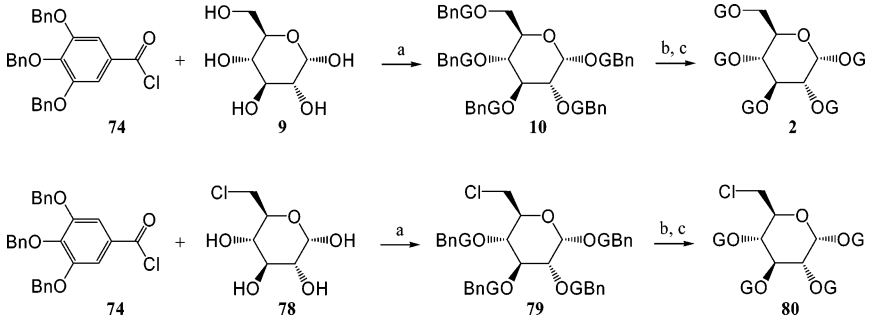
| D-Xylose | | | L-Arabinose | | | D-Ribose | | | D-Lyxose | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| # | α/β | R | # | α/β | R | # | α/β | R | # | α/β | R |
| **58** | α | GBn | **62** | α | GBn | **66** | α | GBn | **70** | α | GBn |
| **59** | α | G | **63** | α | G | **67** | α | G | **71** | α | G |
| **60** | β | GBn | **64** | β | GBn | **68** | β | GBn | **72** | β | GBn |
| **61** | β | G | **65** | β | G | **69** | β | G | **73** | β | G |

**Scheme 2.** Synthesis of 1,2,3,4-Tetra-*O*-galloyl-α-D-glucopyranose **77**[a]



[a] Reagents and conditions: (a) $(COCl)_2$, DMF (cat.), toluene; (b) TrCl, pyridine, 40 °C; (c) **74**, DMAP, $CH_3CN$; (d) HBr, AcOH; (e) chromatography; (f) $H_2$, THF, 10% Pd/C, 40 °C; (g) crystallization.

**Scheme 3.** Selective Synthesis of Derivatives of **2**[a]



[a] Reagents and conditions: (a) DMAP, $CH_3CN$; (b) $H_2$, THF, 10% Pd/C, 40 °C; (c) crystallization.

Compounds **28**−**45** (Chart 3) are analogues containing one galloyl group less than **2** and **3**. Among these compounds, only **43** (6-deoxy-tetra-*O*-galloyl-α-D-glucopyranose) is active. The removal of a galloyl group from position 1, 2, 3, or 4 of glucose resulted in a complete loss of glucose transport stimulation. However, activity was retained when a galloyl group linked to position 6 was removed and the α-configuration at C-1 was present. These results suggest that the galloyl groups linked to

position 1, 2, 3, or 4 of glucose in **2** are functionally important, while the galloyl group bound to C-6 is not. On the other hand, **3** requires all five galloyl groups to induce activity.

The final series of novel hexa- and pentapyranoses, **46**−**76** (Chart 4) tested the dependence of activity on the stereochemical arrangement of the galloyl groups attached to the carbohydrate core. The configuration at one of the positions C-2, C-3, or C-4 of the corresponding α- or *β*-glucopyranose (**2** or **3**) was inverted for **46**−**57** and **61**−**73** and retained for **58**−**61**. The only active compound was found to be 1,2,3,4-tetragalloyl-α-D-xylopyranose **59**. Its structure shows the same configuration at C-2, C-3, and C-4 as found in **2** and **3**. The fact that all other compounds in this series were inactive (Table 1) shows that, among the different monosaccharides tested, the pyranose ring of glucose with its equatorial substituents at C-2, C-3, and C-4 provides the best spatial orientations for the galloyl groups to couple to and to present themselves to the IR in order to induce glucose transport. It indicates that the spatial arrangement of galloyl groups in **2** and **3** greatly impacts glucose transport stimulation.

The observation that **43** and **59** are active indicates that variations of the structure around C-6 of the glucopyranose core of **2** are tolerated. This suggested that it might be possible to enhance the glucose transport activity of **2** by changing the groups bound to C-5 or C-6 of the glucopyranose ring. To test this hypothesis, we developed two novel syntheses (Schemes 2 and 3) to access specifically derivatives of **2** that are modified at C-6. 1,2,3,4-Tetra-*O*-galloyl-α-D-glucopyranose **77** shows an activity very similar to that of **2**. 6-Chloro-6-deoxy-1,2,3,4-tetra-*O*-galloyl-α-D-glucopyranose **80** was found to be ∼30% more active than **2**. Even more remarkable, **80** is as active as insulin (Table 1).

Little was known about the antidiabetic activity of **2** and **3** and their analogues before this study. SAR investigations of gallotannins were only conducted in other biomedical areas. **3** was shown to have higher fatty acid synthase inhibitory activity than other tannin compounds.[39] Gallotannins were found to possess anticancer and anti-inflammatory activities.[40,41] The number of galloyl groups in gallotannins was found to be important for their antiherpetic[42] and antioxidant[43] activities. In addition, the presence of all three unprotected phenolic hydroxyl groups was shown to be crucial for antioxidant activity.[43] Some of our experimental results are consistent with the studies described above.

Our previous mechanistic studies showed that **2** binds to the α-subunit of IR and activates the IR-mediated signaling pathway for glucose transport activity.[38] The present study indicates that at least four galloyl groups of **2** are critical for the activity of the compound. These results lead to the hypothesis that binding of **2** to IR is responsible for the glucose transport stimulatory activity. The glucose core appears to provide a near-optimal spatial arrangement for the galloyl groups to form proper chemical interactions with the amino acid residues of the IR located in the binding site. Our previous studies[38] suggest that the α-subunit of the IR forms the binding site for **2** and its analogues. Since the removal of phenolic hydroxyl groups leads to loss of activity, the interaction may take place primarily through the peripheral hydroxyl groups in the form of hydrogen bonding. The shape and polarity of the residue-linked C-6 may also affect the binding. Currently a more detailed characterization of the binding between **2** and IR is under investigation. Our in vivo studies established that **2** effectively reduces blood glucose and insulin levels in diabetic mice.[38] A preliminary pharmacokinetic study indicates that **2** is orally bioavailable because it can be detected in blood 1 h after delivering it into mice by the gavage method (data not shown). In summary, our data suggest that the active compounds presented in this study may provide the molecular model for the design and development of new antidiabetic leads.

## Conclusion

We have designed and synthesized a series of novel gallotannin analogues by modifying the structure of our lead compounds **2** and **3**. We measured the glucose transport stimulatory activities of these compounds in 3T3-L1 adipocytes. The study shows that both the glucose core and the galloyl substituents with their three phenolic hydroxyl groups are essential for activity. In **2** the glucopyranose ring provides an optimal scaffold for the galloyl groups on C-1, C-2, C-3, and C-4 to present themselves in a conformation needed for the induction of activity. C-6 and the galloyl group attached to it are not crucial for the induction of activity. Our results demonstrate that more potent and efficacious derivatives of **2**, such as **80**, may be accessible by changing the structure around this part of the molecule. On the other hand, **3** was the only activity-inducing compound with an equatorial galloyl group attached to C-1. This study provides the basis for our ongoing efforts to discover new potent insulin mimetics and to elucidate the mechanism of the potentially antidiabetic activities of **2** and **3**.

## Experimental Section

All procedures were carried out using anhydrous solvents purchased from commercial sources and used without further purification. 1-Deoxy-D-glucose, 2-deoxy-D-glucose, and 3-deoxy-D-glucose were purchased from Toronto Research Chemicals, Canada. 4-Deoxy-D-glucose was purchased from CMC Chemicals, England. 6-Deoxy-D-glucose was purchased from Sigma. Ellagic acid (**27**) was purchased from Acros Chemical. Gallic acid (**4**), methyl galloate (**5**), α-D-glucose (**9**), α-D-glucopyranose pentaacetate (**12**), *β*-D-glucopyranose pentaacetate (**13**), α-D-glucopyranose pentabenzoate (**14**), and all other reagents were purchased from Aldrich. Chromatographic separations were performed on silica gel using the solvent systems indicated. Solvent systems are reported as v/v percent ratios. All reactions were monitored by TLC using precoated silica gel plates. Yields refer to chromatographically and spectroscopically pure compounds except when otherwise indicated. Product analysis by HPLC indicated that purities of all synthesized and purchased target compounds were greater than 98%.

Melting points were determined on a Mel-Temp II apparatus (Laboratory Devices) and are uncorrected. Proton nuclear magnetic resonance spectra were recorded at 500 MHz with a Varian INOVA-500 spectrometer, and chemical shifts are expressed in $\delta$ (parts per million, ppm). Coupling constants ($J$) were expressed in Hz. The splitting patterns are abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad singlet). IR spectra were recorded on a Shimadzu Advantage FTIR 8400S spectrometer. Optical rotations were measured on an AUTOPOL IV (Rudolph Research Analytical) polarimeter at 20 °C and 589 nm. Mass spectra were recorded on a Micromass Q-TOF II (Micromass, Wythenshawe, U.K.). Silica gel thin-layer chromatography was performed on precoated Kieselgel 60$F_{254}$ (Merck KGaA, Germany) plates. For column chromatography, silica gel (ACROS Organics) with particles size of 0.035−0.070 mm was used.

HPLC was performed on a Beckman (Beckman Coulter) HPLC instrument with a model 118 pump, model 7725i injector, and a Beckman 168 photodiode array detector. Chromatograms were recorded using System Gold 32 Karat software (Beckman). Solvent A of the mobile phase of was made of deionized water (Ultrapure Water, Dubuque, IA) and trifluoroacetic acid (Fisher Scientific) (999:1). Solvent B consisted of acetonitrile (Sigma-Alderich) and trifluoroacetic acid (999:1). A linear gradient was applied from 80:20 A/B to 50:50 A/B over 15 min at a flow rate of 1 mL/min for the analytical column and at 3 mL/min for the semipreparative

column. Detection wavelength was at 280 nm, and analyses were conducted at 20 °C. Analytical HPLC was carried out on a Beckman Ultrasphere ODS column (250 mm × 4.6 mm i.d., 5 μm) used together with an Ultrasphere ODS precolumn (4 mm × 4.6 mm i.d.) using an injector with a 10 μL loop. Preparative HPLC was carried out on a Beckman Ultrasphere ODS column (250 mm × 10 mm i.d., 5 μm) used together with an Ultrasphere ODS precolumn (4 mm × 4.6 mm i.d.) using an injector with a 100 μL loop.

3T3-L1 preadipocytes were purchased from American Type Culture Collection (ATCC, Rockville, MD). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's PBS (DPBS), fetal bovine serum (FBS), and calf bovine serum (CBS) were purchased from Cellgro, Inc.. Kerbs-Ringer-HEPES (KRP) buffer had the following composition: 136 mM NaCl, 4.7 mM KCl, 1.25 mM $CaCl_2$, 1.25 mM $MgSO_4$, and 10 mM sodium phosphate buffer at pH 7.4. 3-Isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), and bovine insulin were purchased from Sigma Chemical (St. Louis, MO). 2-Deoxy-[1-$^3$H]-D-glucpse (1 mCi/mL) was purchased from Amersham. Radioactive glucose taken up by the cells was measured by a LS 6500 multipurpose scintillation counter from Beckman Coulter.

**3,4,5-Tribenzyloxybenzoate (8).** A mixture of 10.00 g (54.3 mmol) of methyl 3,4,5-trihydroxybenzoate, 4.00 g (24 mmol) of potassium iodide, and 44.00 g (318 mmol) of anhydrous powdered potassium carbonate in 500 mL of acetone was stirred at room temperature for 20 min. Then 22.00 g (174 mmol) of benzyl chloride dissolved in 100 mL of acetone was added. The suspension was refluxed for 18 h. TLC shows full conversion of the starting material and a single product spot ($R_f$ = 0.30; hexanes/ethyl acetate = 8:2). The solid was filtered, and the filtrate was evaporated. The residue was taken up in 400 mL of dichloromethane. The suspension was filtered through Celite, and the filtrate was evaporated. After the mixture was dried for 1 h under vacuum, methyl 3,4,5-tribenzyloxybenzoate **7** was obtained as a white solid that was used for the next step without further purification.

Crude **7** (26.52 g) was suspended in 500 mL of 95% ethanol. An amount of 3.54 g (88.5 mmol) of sodium hydroxide was added. The mixture was heated at reflux for 2 h. The hot solution was poured into 525 mL of 0.6 M hydrochloric acid. A thick, voluminous suspension formed. The hot mixture was stirred for 10 min before the solid was filtered off. The product was washed successively with a 1:1 mix of 95% ethanol and water (100 mL), pure water (100 mL), 95% ethanol (100 mL), methanol (2 × 50 mL), and *tert*-butyl methyl ether (2 × 50 mL). The solid was dried overnight under vacuum to yield 22.6 g (94%) of colorless crystals. Mp 191−194 °C (lit.[51] 196−196.5 °C).

**α- and β-D-Glucopyranose Pentakis[3,4,5-tris(phenylmethoxy)-benzoate] 10 and 11 via Steglich Esterification (Scheme 1).** A suspension of 0.21 g (1.17 mmol) of D-glucose, 3.77 g (8.57 mmol) of 3,4,5-tribenzyloxybenzoic acid, 2.2 g (10.68 mmol) of dicyclohexylcarbodiimide (DCC), and 1.2 g (9.84 mmol) of *N*,*N*-(dimethylamino)pyridine (DMAP) in 135 mL of dry dichloromethane was refluxed for 18 h. After the mixture was cooled to room temperature, the urea byproduct was filtered and the filtrate was evaporated. The resulting residue was purified by column chromatography on silica gel, using a 75:25:1 mixture of dichloromethane, toluene, and ethyl acetate as the eluent. The product fraction was evaporated, and the residue was precipitated from toluene. Compounds **10** (337 mg, 14.7%) and **11** (319 mg, 11.7%) were obtained.

**Compound 10:** white solid, $R_f$ (94% toluene/6% ethyl acetate), 0.55; $[\alpha]^{24}_D$ +43.53° (*c* 0.17, $CH_2Cl_2$); IR (KBr) 1724, 1589, 1499 cm$^{-1}$; HRMS (ESI) ($C_{146}H_{122}O_{26}$), *m*/*z* calculated M + $Na^+$ 2313.8122, measured M + $Na^+$ 2313.8152 (1.2 ppm); $^1$H NMR ($CDCl_3$) $\delta$ 4.36 (1H, dd, $J$ = 6.0, 12.5), 4.64 (1H, m), 4.83 (1H, dd, $J$ = 2.5, 12.5), 4.89−5.16 (30H, m), 5.65 (1H, dd, $J$ = 3.5, 10.0), 5.81 (1H, t, $J$ = 10.0), 6.40 (1H, t, $J$ = 10.0), 6.88 (1H, d, $J$ = 3.0), 7.20−7.53 (85H, m).

**Compound 11:** white solid, $R_f$ (94% toluene/6% ethyl acetate), 0.57; $[\alpha]^{24}_D$ −0.59° (*c* 0.17, $CH_2Cl_2$); IR (KBr) 1727, 1589, 1499 cm$^{-1}$; HRMS (ESI) ($C_{146}H_{122}O_{26}$), *m*/*z* calculated M + $Na^+$ 2313.8122, measured M + $Na^+$ 2313.8203 (3.5 ppm); $^1$H NMR ($CDCl_3$) $\delta$ 4.41 (1H, dd, $J$ = 6.5, 12.5), 4.49 (1H, m), 4.81 (1H, m), 4.96−5.20 (30H, m), 5.79 (1H, t, $J$ = 10.0), 5.88 (1H, dd, $J$ = 8.0, 10.0), 6.10 (1H, t, $J$ = 10.0), 6.26 (1H, d, $J$ = 8.0), 7.20−7.50 (85H, m).

**α-D-Glucopyranose Pentakis[3,4,5-tris(phenylmethoxy)benzoate] (10) via Esterification with Acid Chloride 74 (Scheme 3).** A suspension of 459 mg (1.0 mmol) of acid chloride **74** and 36 mg (0.2 mmol) of finely powdered D-glucose in 10 mL of acetonitrile was stirred at room temperature. Then an amount of 128 mg (1.05 mmol) of DMAP was added, and the mixture was stirred at room temperature. After 18 h, the solvent was evaporated. The residue was taken up in 5 mL of toluene at 60 °C. After the mixture was cooled to room temperature, the solution was filtered through a layer of silica gel (250 mg, 1.2 cm thick). The silica gel was washed with 5 mL of a mix of toluene and ethyl acetate (100:4). The solvent was evaporated. An amount of 455 mg (99%) of **74** was obtained as a highly viscous oil after drying in a vacuum for 5 h (for analytical data, see above).

**α-D-Glucopyranose Pentakis(3,4,5-trihydroxybenzoate) (2) (α-PGG).** A suspension of 332 mg (0.145 mmol) of **10** and 31.8 mg (0.30 mmol) of palladium (10 wt % on activated carbon) in 30 mL of dry THF was stirred at 40 °C under a hydrogen gas atmosphere for 16 h. The reaction mixture was cooled and filtered through Celite filter-aid, and the filtrate was evaporated. The residue was crystallized from water. Compound **2** (85.0 mg, 62.2%) was obtained as colorless crystals. Mp >210 °C (dec); $[\alpha]^{24}_D$ +167.06° (*c* 0.17, $CH_3COCH_3$); IR (KBr) 3390, 1699, 1612, 1535 cm$^{-1}$; HRMS (ESI) ($C_{41}H_{32}O_{26}$), *m*/*z* calculated M − $H^+$ 939.1104, measured M − $H^+$ 939.1107 (0.3 ppm); $^1$H NMR ($CD_3COCD_3$) $\delta$ 4.38 (1H, dd, $J$ = 4.0, 13.0), 4.5 (1H, dd, $J$ = 1.5, 11.0), 4.66 (1H, d, $J$ = 9.5), 5.45 (1H, dd, $J$ = 3.5, 9.0), 5.76 (1H, t, $J$ = 10.5), 6.16 (1H, t, $J$ = 10.5), 6.72 (1H, d, $J$ = 3.5), 6.90 (2H, s), 6.91 (2H, s), 7.02 (2H, s), 7.18 (2H, s), 7.22 (2H, s).

**β-D-Glucopyranose Pentakis(3,4,5-trihydroxybenzoate) (3) (β-PGG).** A suspension of 314 mg (0.137 mmol) of **11** and 31.8 mg (0.30 mmol) of palladium (10 wt % on activated carbon) in 30 mL of dry THF was stirred at 40 °C under a hydrogen gas atmosphere for 16 h. The reaction mixture was cooled and filtered through Celite filter-aid, and the filtrate was evaporated. The residue was dissolved in water at 60 °C. When the mixture was cooled, compound **3** precipitated. After filtration and drying under vacuum for 20 h, an amount of 79.0 mg (61.7%) of a white solid was obtained. $[\alpha]^{24}_D$ +17.14° (*c* 0.14, $CH_3COCH_3$); IR (KBr) 3392, 1708, 1616, 1535 cm$^{-1}$; HRMS (ESI) ($C_{41}H_{32}O_{26}$), *m*/*z* calculated M − $H^+$ 939.1104, measured M − $H^+$ 939.1075 (3.1 ppm); $^1$H NMR ($CD_3COCD_3$) $\delta$ 4.36 (1H, dd, *J* 5.0, 13.0), 4.52 (1H, m), 4.54 (1H, m), 5.60 (1H, t, *J* 9.5), 5.64 (1H, t, *J* 10.0), 5.99 (1H, t, *J* 10.0), 6.30 (1H, d, *J* 8.5), 6.95 (2H, s), 6.99 (2H, s), 7.04 (2H, s), 7.09 (2H, s), 7.16 (2H, s).

**3,4,5-Tribenzyloxybenzoyl Chloride (74).** A suspension of 11.57 g (26.26 mmol) of 3,4,5-tribenzyloxybenzoic acid **8** in 120 mL of toluene and 0.2 mL of DMF was stirred at room temperature. Via a dropping funnel, a solution of 5 g (39.4 mmol) of oxalyl chloride in 17 mL of toluene was added over a period of 10 min. After being stirred at room temperature for an additional 20 min, the mixture was heated to 50 °C. The ceasing of gas evolution indicated the end of the reaction after about 1 h. The yellow solution was evaporated. The residue was taken up in 50 mL of toluene at 70 °C. A yellow, highly viscous, sticky side product remained undissolved. The product solution was decanted. The chloride was precipitated by adding 55 mL of cyclohexane to the hot toluene solution. Crystallization started within a few minutes. The solid was filtered and washed with cyclohexane. The product, 9.46 g (78%), was obtained as a white solid. Mp 116−117 °C (lit.[52] 115−116 °C).

**α-D-Glucopyranose 1,2,3,4-Tetrakis(3,4,5-tri-*O*-benzyl-galloate) (76).** A solution of 500 mg (2.78 mmol) of α-D-glucopyranose and 836 mg (3.00 mmol) of trityl chloride in 6 mL of dry pyridine was stirred for 8 h at 40 °C. The mixture was cooled

to room temperature, and 5.35 g (11.66 mmol) of 3,4,5-tribenzyloxybenzoyl chloride (**74**), 1.9 g (15.55 mmol) of DMAP, and an additional 12 mL of pyridine were added. The mixture was stirred for 2 days at room temperature. Solid material was filtered off and washed with 20 mL of toluene. The filtrate was evaporated to dryness and redissolved in toluene, and the solution was evaporated again. The dissolving and evaporation was repeated once more. The residue was purified by column chromatography on silica gel with an eluent consisting of toluene and ethyl acetate (100:1). An amount of 4.75 g of a clear, colorless, highly viscous oil was obtained. The NMR spectrum showed a mixture of α and $\beta$ products (1:1).

The mixture was dissolved in 100 mL of acetic acid at 70 °C. After the mixture was cooled to room temperature, a suspension formed. An amount of 4.3 mL (23.74 mmol) of a 33% solution of hydrogen bromide in acetic acid was added. After exactly 2 min, an amount of 90 mL of ice-cold water was added. It was extracted with dichloromethane (3 × 150 mL). The combined extracts were washed with ice-cold water (3 × 150 mL) and with 100 mL of brine. After the mixture was dried over sodium sulfate, the solvent was evaporated. The residue was purified by column chromatography on silica gel using a mixture of toluene and ethyl acetate (100:8) as the eluent. The product (764 mg, 0.41 mmol, 15%) was obtained as a clear, colorless, highly viscous oil. $[\alpha]^{25}_D$ +47.70° (*c* 1.00, $CH_3COCH_3$); IR (KBr) 1724, 1589, 1499 $cm^{-1}$; HRMS (ESI) $C_{118}H_{100}O_{22}$, *m/z* calculated M + $H^+$ 1891.6604, measured M + $H^+$ 1891.6671 (3.5 ppm); $^1H$ NMR ($CDCl_3$) $\delta$ 3.68 (1H, dd, $J$ = 2.9, 13.2 Hz), 3.78 (1H, dd, $J$ = 1.4, 13.2 Hz), 4.08 (1H, d, $J$ = 10.1 Hz), 4.72−5.08 (24 H, m), 5.52 (1H, t, $J$ = 10.1 Hz), 5.55 (1H, dd, $J$ = 3.5, 10.1 Hz), 6.31 (1H, t, $J$ = 10.1 Hz), 6.77 (1H, d, $J$ = 3.6 Hz), 7.05−7.42 (68H, m).

**1,2,3,4-Tetra-*O*-galloyl-α-D-glucopyranose (77).** A suspension of 300 mg (0.160 mmol) of **77** and 30 mg (0.29 mmol) of palladium (10 wt % on activated carbon) in 15 mL of dry THF was stirred at 40 °C under a hydrogen gas atmosphere for 16 h. The reaction mixture was cooled and filtered through Celite filter-aid, and the filtrate was evaporated. The residue was crystallized from water. Compound **77** (81 mg, 64%) was obtained as colorless crystals. Mp >203 °C (dec); white crystals; $[\alpha]^{25}_D$ +142.55° (*c* 0.55, $CH_3COCH_3$); IR (KBr) 3400, 1723, 1612, 1539 $cm^{-1}$; HRMS (ESI) $C_{34}H_{28}O_{22}$, *m/z* calculated M − $H^+$ 787.0994, measured M − $H^+$ 787.0992 (0.3 ppm); $^1H$ NMR ($CD_3COCD_3$) $\delta$ 3.57−3.67 (2H, m), 4.19 (1H, ddd, $J$ = 2.4, 3.9, 10.0 Hz), 5.31 (1H, dd, $J$ = 3.6 and 10.3 Hz), 5.49 (1H, t, $J$ = 10.0 Hz), 6.02 (1H, t, $J$ = 10.0 Hz), 6.58 (1H, d, $J$ = 3.6 Hz), 6.87 (2H, s), 6.89 (2H, s), 6.96 (2H, s), 7.14 (2H, s).

**6-Chloro-6-deoxy-1,2,3,4-tetra-*O*-galloyl-α-D-glucopyranose (80).** A suspension of 7.133 g (15.5 mmol) of acid chloride **74** and 0.771 g (3.89 mmol) of 6-chloro-6-deoxy-α-D-glucopyranose[48] **78** in 150 mL of acetonitrile was stirred at room temperature. Finally, 1.99 g (16.3 mmol) of DMAP was added, and the mixture is stirred at room temperature. After 18 h, the solvent was evaporated. The residue was suspended in 100 mL of toluene at 60 °C. A total of 5 g of silica gel was added. After an additional 10 min of stirring, the mixture was cooled to room temperature and then filtered through a layer of silica gel (10 g, ∼1.5 cm thick). The filtrate was evaporated, and the residue was dried in high vacuum. Crude **79** was obtained as a highly viscous, colorless oil.

The intermediate **79** was dissolved in 200 mL of dry THF. A total of 0.7 g (0.66 mmol) of 10% palladium on carbon was added. The suspension was stirred vigorously at 40 °C under a hydrogen gas atmosphere at normal pressure for 18 h. The mixture was cooled and filtered through Celite, and the filtrate was evaporated. A slightly pink, highly viscous oil was obtained. It dissolved in 20 mL of water. The mixture was evaporated at <45 °C to remove residual organic solvent. Once more, the residue was taken up in 20 mL of water and dried by evaporation. Again, the residue was dissolved in 20 mL of water. The solution was left at room temperature. After 8 days, the colorless crystals were filtered off, washed with cold water, and dried in a vacuum. An amount of 1.845 g (58%) of **80** was obtained. $[\alpha]^{24}_D$ +111.67° (*c* 0.12, $CH_3COCH_3$); IR (KBr) 3428, 1714, 1617, 1534 $cm^{-1}$; HRMS (ESI) $C_{34}H_{27}O_{21}Cl$, calculated M − H 805.0655, measured M − H 805.0623 (4.0 ppm); $^1H$ NMR ($CD_3COCD_3$) $\delta$ 3.78 (1H, dd, $J$ = 5.0, 12.5 Hz), 3.86 (1H, dd, $J$ = 2.5, 12.5 Hz), 4.61 (1H, m), 5.45 (1H, dd, $J$ = 4.0, 10.5 Hz), 5.63 (1H, t, $J$ = 10.0 Hz), 6.12 (1H, t, $J$ = 9.5 Hz), 6.71 (1H, d, $J$ = 3.5 Hz), 6.98 (2H, s), 7.07 (2H, s), 7.26 (2H, s).

**Glucose Uptake Assay.** 3T3-L1 preadipocytes grown in 24-well plates were induced by MDI (IBMX-DEX-insulin) into adipocytes, which were washed twice and incubated with 0.5 mL of serum-free DMEM in 10% $CO_2$ at 37 °C for 2 h. The cells were washed with 0.45 mL of KRP buffer three times and then incubated at 37 °C for 30 min. Insulin was added to the cells at a final concentration of 1 $\mu$M. Alternatively, **2** or **3** or their analogues were added to the cells at a final concentration of 30 $\mu$M. The adipocytes were incubated at 37 °C for 15 min. Glucose uptake was initiated by addition of 0.1 mL of KRP buffer with 1 $\mu$Ci/mL [1-$^3$H]-2-deoxy-D-glucose and 1 mM cold glucose as the final concentration. The glucose uptake was terminated with addition and then removal of ice-cold PBS buffer. Finally, the cells were lysed with 0.45 mL of 1% Triton X-100 at 37 °C for 20 min. The radioactivity taken up by the cells was measured in a solution of 0.4 mL of the cell lysates and 5 mL of scintillation liquid using a scintillation counter. Assay data were analyzed statistically using the Student's *t*-test by comparison of experimental samples or of positive samples (insulin) with the negative control. To compensate for the multiple *t*-test, $P < 0.01$ was set as the level of significant difference. In the assay, insulin-treated samples served as positive controls while untreated samples were used as negative controls. The assay for each compound was performed twice, and each condition was in duplicate. A compound with the activity statistically significantly higher than that of the negative control was considered active.

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**Supporting Information Available:** Synthetic procedures and analytical data for the remaining analogues of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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