# Regioselective Enzymatic Glycosylation of Natural Polyhydroxylated Compounds: Galactosylation and Glucosylation of Protopanaxatriol Ginsenosides[1]

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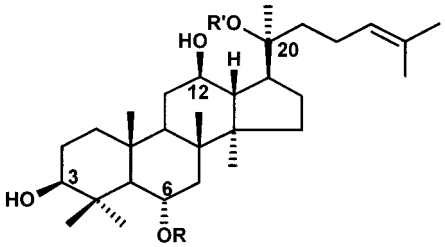
Ginsenoside $Rg_1$ (**1**), the most representative Ginsenoside from *Panax Ginseng* C. A. Meyer belonging to the protopanaxatriol family, has been galactosylated by action of the $\beta$-(1,4)-galactosyltransferase (GalT) from bovine colostrum, using UDP-galactose as an activated sugar donor. The enzyme showed the well-known specificity for the formation of a $\beta$-linkage with the C-4 OH of the glucose acceptor, but it was not able to discriminate between the two glucose moieties of **1**, giving a mixture of mono- and digalactosylated derivatives. Other natural $Rg_1$-analogues such as F1, $Rh_1$, Re, as well as the synthetic derivative 6′-*O*-acetyl-$Rg_1$ have been also galactosylated, giving monolactosyl derivatives. GalT was also able to accept UDP-glucose as an activated sugar donor, giving rise to cellobiosyl derivatives of $Rg_1$.

## Introduction

Exploitation of the water extracts of the dried roots and leaves of *Panax ginseng* C. A. Meyer as an effective "tonic" has been in use in the traditional Chinese medicine for at least some 2000 years,[2] and, following a long scientific discussion, it is now generally accepted that the major active principles of *P. ginseng* extracts are ginsenosides,[3] glycosylated derivatives of the triterpene dammarane structure. As a consequence, the isolation of the pure individual ginsenosidic components as well as the synthesis of their natural and nonnatural derivatives is of paramount importance to increase the knowledge of the pharmacological properties of these extracts.

About 30 different glycosides have been isolated from the roots of *P. ginseng* so far,[2,4] and among them ginsenoside $Rg_1$ (**1**, $Rg_1$, 6,20-di-*O*-glucopyranosyl-20(*S*)-protopanaxatriol) and its derivatives are among the main components. In a first report on the enzymatic elaboration of these glycosides, some years ago we described an efficient preparation of the naturally occurring 6′-*O*-acetyl and 6′-*O*-carboxyacetyl derivatives of **1** via a regioselective acylation catalyzed by the lipase B from *Candida antarctica* suspended in *t*-AmOH.[5]




Our general interest in the enzymatic modification of natural compounds is not limited to the use of hydrolases in organic solvents.[6] For instance, taking advantage of the report by Kren and co-workers on the glycosylation of an ergot alkaloid by action of the $\beta$-1,4-galactosyltransferase from bovine colostrum (GalT),[7] we have used the same reaction protocol for the galactosylation of the alkaloid colchicoside,[1] of the coumarinic glucoside fraxin,[1] and of the noncaloric sweetener stevioside.[8] With the latter substrate, GalT proved to be extremely regioselective, only one of the three glucose moieties of this molecule being galactosylated. These results showed us that this enzymatic methodology is very attractive for the selective modification of complex glycosides, and to broaden its application, we examined the behavior of

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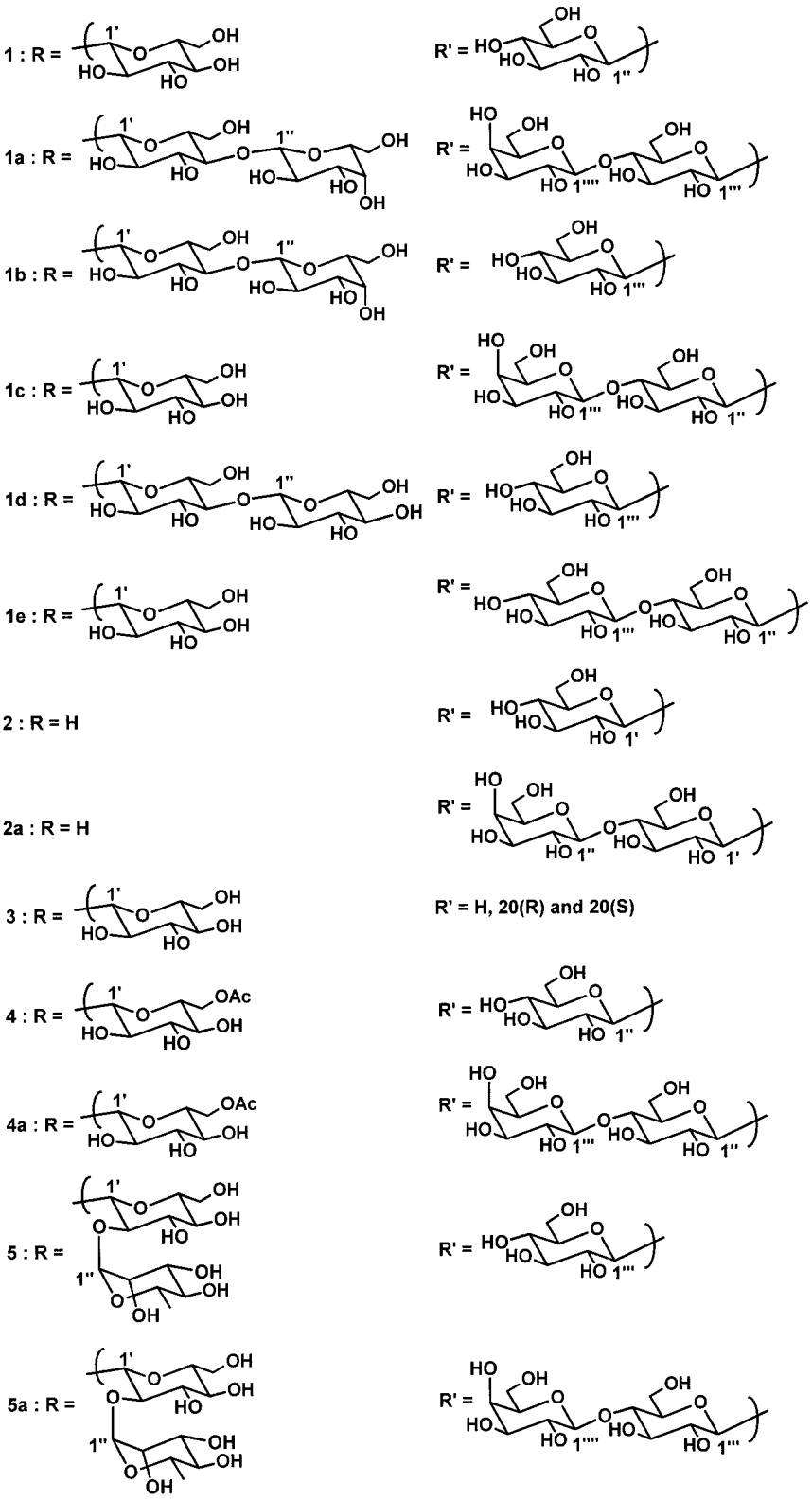
**Chart 1**




GalT toward glucosylated ginsenosides. We report here on the results obtained for the glycosylation of ginsenoside $Rg_1$ (**1**) and of other related compounds (**2**−**5**) belonging to the protopanaxatriol family (Chart 1).

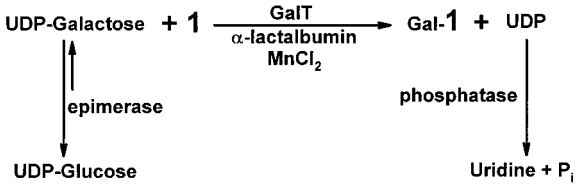
## Results and Discussion

The use of the glycosyltransferases of the so-called "Leloir pathway" for the synthesis of nonnatural oligosaccharides is a well-assessed—even if not so widely used—synthetic tool nowadays.[9] The recent progress in cloning techniques is supplying an ever growing number of new transferases,[9,10] while optimization of the reaction protocols allows the in situ regeneration of the costly sugar nucleosides.[11] However, despite these improvements, only a limited number of the more than 100 glycosyltransferases described so far is commercially available for

preparative use. Among them, the $\beta$-(1−4)-galactosyltransferase(GalT),[12] at first extracted from bovine colostrum or milk, is the most widely studied and exploited enzyme, mainly because of its relative natural abundance. This enzyme has been largely used to support the synthesis of complex branched chain oligosaccharides.[13] Concerning the enzymatic modification of sugar derivatives, attention has been paid to glycopeptides[14] and glycoproteins,[15] while very few examples have been reported so far on the glycosylation of glycolipids[16] or of glycosides of alkaloids,[7] polyketides[1] and terpenes.[8]

Scheme 1 shows the simplified multienzymatic approach that we have used for the GalT-catalyzed galactosylation of ginsenoside $Rg_1$. The expensive UDP-galactose was prepared in situ from the cheaper UDP-glucose by action of an UDP-glucose epimerase (epimerase), while the overall equilibrium was driven to the right by the hydrolytic cleavage of UDP catalyzed by an alkaline phosphatase (phosphatase). $Mn^{2+}$ is an essential cofactor for GalT, while α-lactalbumin is needed when the sugar acceptor is glucose or a glucose derivative.

**Scheme 1. Multienzymatic Protocol Used to Galactosylate Ginsenoside $Rg_1$ (1)**




A 40 mM solution of **1** in 50 mM Tris buffer pH 7.4, containing 2 equivalents of UDP-glucose and the above-described enzymatic cocktail (GalT, epimerase, phosphatase) was allowed to react at 30 °C for 4 days, giving the reaction outcome depicted by the HPLC-chromatogram of Figure 1 (73% conversion, see Table 1). The compounds corresponding to the three main HPLC peaks (compound A, $t_R$ 12.05; compound B, $t_R$ 18.83; compound C, $t_R$ 28.19; ginsenoside $Rg_1$ has $t_R$ 43.44) were isolated by preparative RP-HPLC. Two additional reaction products were also present (compound D, $t_R$ 13.84 and compound E, $t_R$ 32.76, respectively), but, due to their low amount, they could not be isolated at this stage.

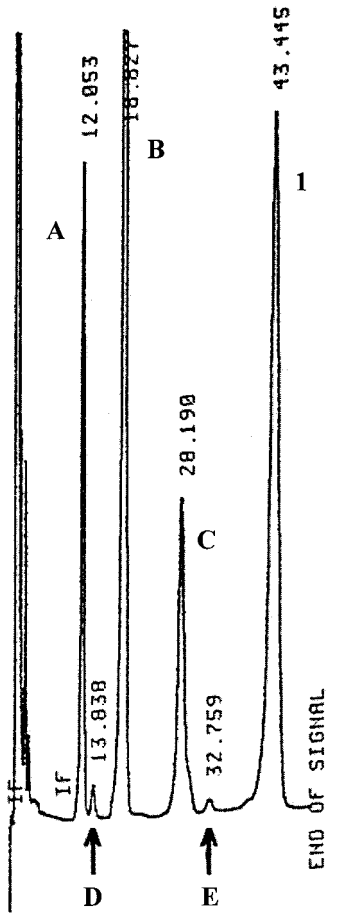



**Figure 1.** HPLC chromatogram of the GalT-catalyzed glycosylation of ginsenoside $Rg_1$ (**1**).

**Table 1. GalT-Catalyzed Glycosylation of Protopanaxatriol Ginsenosides**

| substrate | DMSO (% v/v) | reaction time, h | % conversion[a] | product | % isolated yields |
|---|---|---|---|---|---|
| **1** | 0 | 96 | 73 | **1a** | 11 |
| | | | | **1b** | 26 |
| | | | | **1c** | 13 |
| **1** | 10 | 24 | 97 | **1a** | 32 |
| | | | | **1b** | 31 |
| **2** | 20 | 72 | 62 | **2a** | 51 |
| **4** | 20 | 72 | n.d. | **4a** | 36 |
| **5** | 20 | 48 | n.d. | **5a** | 41 |

[a] Determined by HPLC.

To perform the structural characterization of the three isolated reaction products, the MS spectra were registered first in order to evaluate the number of added glycosyl moieties.[17] Then, a detailed investigation of the NMR spectra (registered with 1D- and 2D techniques) allowed the determination of the linkage position of the enzymatically introduced galactopyranosyl moiety(ies), and their anomeric configuration.

The most abundant reaction product, compound B, was analyzed first. In its negative ion LSIMS spectrum a quasimolecular ion peak ($[M - H]^-$) was observed at *m/z* 961, indicating the presence of one additional hexose moiety with respect to $Rg_1$ ($[M-H]^-$ at *m/z* 799). For reasons of solubility, its NMR spectra were run in $CD_3OD-Py-d_5$ (5:1), this solvent mixture giving nicely well-

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resolved signals in the proton domain. The resonances of H-3, H-6, and H-12 were assigned by inspection of chemical shift and multiplicity, those of C-3, C-6, and C-12 by HMQC experiments, while the C-20 signal was easily detected as the most downfield shifted oxymethine singlet. The signals of the anomeric protons of the glucose moieties linked at C-6 (C6-Glcp) and C-20 (C20-Glcp) were detected at $\delta$ 4.55 and at $\delta$ 4.75 ppm, respectively (HMBC experiments). Another signal due to a $\beta$-oriented anomeric proton appeared at $\delta$ 4.60 ppm ($J$ = 7.8 Hz), due to the new sugar unit. This additional hexose was linked at the C-4 OH of the C6-Glcp. In fact, this C-4 signal was downfield shifted from $\delta$ 71.26 ppm to $\delta$ 81.60 ppm and showed a cross-peak in the HMBC spectrum to the proton signal at $\delta$ 4.60 ppm. The corresponding H-4 signal appeared at $\delta$ 3.77 ppm (t, $J$ = 7.8 Hz) and was shown to be connected to H-1 at $\delta$ 4.55 ppm via H-3 at $\delta$ 3.75 and H-2 at $\delta$ 3.54 (TOCSY and COSY experiments). In a similar way, the H-1 signal of the newly introduced sugar (at $\delta$ 4.60 ppm) was correlated to its equatorial H-4 at $\delta$ 4.01 ppm (d, $J$ = 3.2 Hz) via H-2 at $\delta$ 3.90 and H-3 at $\delta$ 3.68 ppm, thus confirming the galactopyranosyl nature of this monosaccharide. Thus, the structure of the most abundant compound B is that of ginsenoside $Rg_1$ monogalactosylated at the C-4 position of the "lower" glucose, namely that of 6-*O*-lactosyl-20-*O*-glucosyl protopanaxatriol (**1b)**.

Following a similar structure elucidation protocol, the most retained product (compound C, $[M - H]^-$ at $m/z$ 961) was shown to be the 6-*O*-glucosyl-20-*O*-lactosyl protopanaxatriol (**1c)**. In this case, the anomeric proton of C20-Glcp at $\delta$ 4.76 ppm was correlated to its H-4 at $\delta$ 3.78 ppm. The corresponding C-4 signal resulted downfield shifted ($\delta$ 81.12 ppm) because of its linkage to a galactopyranoside unit, which in turn showed H-1 at $\delta$ 4.62 ppm ($J$ = 7.8 Hz) and an equatorial H-4 signal at 4.03 ppm (d, $J$ = 3.2 Hz).

Finally, the structure of the less retained product (compound A, $[M - H]^-$ at $m/z$ 1123) resulted that of the 6,20-di-*O*-lactosylprotopanaxatriol (**1a)**, as expected. Unfortunately, the analysis of the proton spectrum of this compound in the $CD_3OD-Py$-$d_5$ mixture was hampered by extensive signals overlapping, and the data of **1b** and **1c** could not be used for comparison. After several attempts with different solvents, we could obtain an acceptable signals resolution by using neat $CD_3OD$. As a consequence, a parallel spectroscopic investigation of **1a** was performed in this solvent, in comparison with the parent compound **1** in the same solvent, and data are detailed in the Experimental Section.

Therefore, at variance with our previous observations concerning the GalT-catalyzed galactosylation of the polyglucosylated terpene stevioside,[8] both the "upper" and the "lower" glucopyranosyl moieties of **1** were sugar acceptor substrates for GalT. However, these two sugars were not similarly recognized by this enzyme. In fact, when the enzymatic reaction was performed in the presence of a large excess of sugar donor (5 equivalents of UDP-glucose) and using 10% v/v of DMSO (to allow the complete solubility of the substrates), a 97% conversion was observed after 24 h to only two products, **1a** and **1b**, which were isolated in 32% and 31% yield, respectively. This result indicates that the glucose moiety linked at C-20 is the less accepted substrate, so that the corresponding derivative **1c**, once formed, is rapidly galactosylated to give the dilactoside **1a**.

The different reactivity of the two glucopyranosyl moieties of **1** was further confirmed by comparing the galactosylation rates of the two complementary monoglucosylated derivatives ginsenoside F1 (**2**), prepared by hesperidinase-catalyzed hydrolysis of **1**,[4] and ginsenoside $Rh_1$ (**3)**, obtained as a mixture of C-20 epimers by acid hydrolysis of **1**.[18] After 24 h, HPLC analysis of analytical samples, obtained under similar reaction conditions, showed a 88% conversion of **3** and only a 16% conversion of **2**. Unfortunately, the poor solubility of **3**, even in the presence of cosolvents, prevented the isolation of the product on a preparative scale. LCMS analysis of the reaction product gave a $[M - H]^-$ peak at $m/z$ 799, thus confirming that **3** was converted into a glycosylated product. On the other hand, preparative scale galactosylation of **2** gave the expected 20-*O*-lactosyl protopanaxatriol **2a** (Table 1, third line), fully characterized as shown in the Experimental Section.

Finally, the influence of substituents on the enzymatically preferred C-6 glucose moiety was investigated by analyzing the reaction outcome of the galactosylation of 6′-*O*-acetyl ginsenoside $Rg_1$ (**4**)[5] and of ginsenoside Re (**5**). Both compounds, carrying, respectively, an acetyl and a rhamnose moiety on the C6-Glcp of the dammarane skeleton, were substrates for GalT, and a single product was isolated (Table 1). However, in both cases, galactosylation took place exclusively on the C20-Glcp, giving rise to compounds **4a** and **5a**, respectively (for structural characterization see the Experimental Section). This finding indicates that structural modification of the C6-Glcp makes this sugar moiety, previously the more reactive one, not available any more for the GalT active site.

In the last step of our investigation, we questioned the nature of the two small peaks indicated by the arrows in the chromatogram of Figure 1 and named compound D and compound E. It has been reported that GalT can accept UDP-glucose as a poor sugar donor,[19] indicating that, using *N*-acetylglucosamine as acceptor, the activity was approximately only 0.3% of that observed with the natural substrate UDP-galactose.[19a,b] More recently, Kren, Augé and co-workers reported that the ergot alkaloid elymoclavine 17-*O*-(2-acetamido)-2-deoxy-$\beta$-D-glucopyranoside could be converted into the corresponding *N*-acetylcellobiosamine derivative in the absence of UDP-glucose epimerase.[7] In another paper, Kren and coworkers described similar results in the glucosylation of chitoligomers.[20] Therefore, we reasoned that the small peaks of Figure 1 might be due to glucosylated $Rg_1$ products. To verify this hypothesis, we submitted **1** to the action of the GalT in the presence of α-lactalbumin and $MnCl_2$, but without adding UDP-glucose epimerase. A 30% conversion was observed after 4 days, and compounds D and E could be isolated by preparative HPLC. Compounds D and E gave the same LSIMS spectra, showing a quasimolecular peak $[M - H]^-$ at $m/z$ 961, indicating the presence of one additional hexose moiety. In the $^1H$ NMR spectrum of compound D, the

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signals of the anomeric protons of the C6-Glcp and the C20-Glcp were found at $\delta$ 4.38 and 4.59 ppm. The anomeric proton of the new sugar unit appeared $\beta$-oriented as a doublet at $\delta$ 4.40 ppm ($J$ = 7.8 Hz) and the HMBC spectrum showed its coupling to the downfield shifted C-4 signal at $\delta$ 80.97 ppm of the C6-Glcp. The glucosyl nature of the introduced sugar was proved by the analysis of the complete spin system using TOCSY and COSY experiments, which showed that the anomeric proton at $\delta$ 4.40 ppm was linked via H-2 at $\delta$ 3.21 ppm and H-3 at $\delta$ 3.35 ppm to an axial H-4 at $\delta$ 3.54 ppm. Thus, the structure of compound D was proved to be 6-*O*-cellobiosyl-20-*O*-glucosylprotopanaxatriol (**1d**). Similarly, Compound E was identified as 6-*O*-glucosyl-20-*O*-cellobiosylprotopanaxatriol (**1e**).

## Conclusions

The results reported in this paper confirm that glycosylations catalyzed by $\beta$-(1,4)-GalT are not restricted to simple sugars, but can be applied to more complex saccharides, provided that a nonsubstituted glucopyranosyl moiety is present in the molecule. The enzyme showed the well-known specificity for the C-4-OH of the sugar substrate but, contrary to what was previously observed with stevioside,[8] both the glucose moieties of **1** were glycosylated. Presently, the results obtained with compounds **1**−**5** are not easily rationalizable, despite the fact that the crystal structure of the catalytic domain of bovine GalT and of its complex with UDP-galactose has been reported recently.[21] In fact, neither information on α-lactalbumin and on sugar acceptor binding sites nor even a complete characterization of the binding of UDP-galactose to GalT are presently available. As a consequence, the possibility of a correct analysis of the interaction of the substrates with the enzyme active site by molecular modeling is still lacking. Despite this, the data reported here further exemplifies the efficiency of biocatalysis for the mild and selective elaboration of natural compounds,[22] allowing one-step transformations that are presently very difficult—if not impossible—to be performed using chemical methods.

Finally, the possibility to generate structural diversity by directly grafting a new hexose to an existing glucose of a complex natural glycoside is of pharmacological interest. In fact, it is known that galactosylation can modulate the pharmacokinetics and biodistribution of these compounds by enhancing the hepatocyte uptake [23] and liver targeting may have wide therapeutic implications due to the numerous postulated liver-related modes of actions of ginsenosides.

## Experimental Section

**Materials and Methods.** UDP-glucose, α-lactalbumin from bovine milk, UDP-galactose-4′-epimerase (EC 5.1.3.2, from galactose-adapted yeast), and alkaline phosphatase (EC 3.1.3.1, from bovine intestinal mucose, type VII S) were from Sigma. $\beta$-1,4-Galactosyltransferase (EC 2.4.1.22, from bovine colostrum) was either purchased from Sigma or purified as described elsewhere,[24] and its activity was evaluated with a spectrophotometric assay.[25] HPLC analyses were performed using a JASCO HPLC instrument (model 880-PU pump, model 870-UV/vis detector) and a Licrospher 100 RP-18 (5 $\mu$m, Merck) reversed-phase analytical column. HPLC purifications were performed using a Partisil 10 ODS-3 column (Whatman). Mass spectra were recorded with a Finnigan MAT 8500 (4.5 kV Cs beam, negative ion mode, glycerol as matrix), and the LCMS experiment was performed with a LCQ Instrument (Finnigan MAT). NMR spectra were taken with Bruker AC 200, AMX 500, and Varian Inova 400 and Inova 600 (5 mm probe head) instruments. H−H COSY: 45° mixing pulse. TOCSY: phase-sensitive mode using TPPI, mixing time 100 ms (100 MLEV-17 cycles plus two trim pulses of 2.5 ms each). HMQC: phase-sensitive mode using TPPI, BIRD sequence, GARP decoupled. HMBC: phase-sensitive mode using TPPI, delay tuned to long-range couplings, 71 ms. Melting points were determined using a Kofler apparatus and are uncorrected. TLC: precoated silica gel 60 $F_{254}$ plates (Merck). Flash chromatography: silica gel 60 (70−230 mesh, Merck).

**Ginsenoside $Rg_1$ (1)** was isolated by column chromatography from a root extract of Korean Ginseng supplied by Indena, Milano (Italy): selected $^1$H NMR data (500.1 MHz, $CD_3OD$) $\delta$ 0.95 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.09 (3H, s), 1.32 (3H, s), 1.34 (3H, s), 1.62 (3H, s), 1.67 (3H, s), 3.07 (1H, t, $J$ = 7.8 Hz, H-2″), 3.09 (1H, dd, $J$ = 11.6, 4.9 Hz, H-3), 3.19 (1H, t, $J$ = 7.8 Hz, H-2′), 3.20 (1H, m, H-5″), 3.27 (1H, m, H-5′), 3.29 (1H, m, H-4″), 3.30 (1H, m, H-4′), 3.33 (1H, t, $J$ = 7.8 Hz, H-3′), 3.35 (1H, t, $J$ = 7.8 Hz, H-3″), 3.62 (1H, m, H-6a″), 3.63 (1H, m, H-6a′), 3.67 (1H, td, $J$ = 10.4, 5.3 Hz, H-12), 3.77 (1H, dd, $J$ = 11.8, 2,3 Hz, H-6b″), 3.81 (1H, dd, $J$ = 11.8, 2.0 Hz, H-6b′), 4.08 (1H, td, $J$ = 10.2, 3.1 Hz, H-6), 4.34 (1H, d, $J$ = 7.8 Hz, H-1′), 4.59 (1H, d, $J$ = 7.8 Hz, H-1″), 5.10 (1H, bt, $J$ = 6.9 Hz, H-24); $^{13}$C NMR (50.3 MHz) $\delta$ 16.11, 17.12, 17.66, 17.81, 17.94, 22.83, 24.24, 25.84, 27.26, 27.61, 30.99, 31.40, 31.55, 36.67, 40.21, 40.40, 40.51, 41.91, 45.33, 49.43, 50.62, 52.46, 53.15, 61.82, 62.59, 62.96, 71.26, 71.76, 71.88, 75.42, 75.53, 77.68, 77.96, 78.29, 79.11, 79.89, 80.93, 84.94, 98.32, 105.58, 125.87, 132.30; LSIMS $m/z$ 799 $[M - H]^-$, 637, 475.

**Enzymatic Galactosylation of Ginsenoside $Rg_1$.** (a) GalT (1 U), epimerase (7 U), alkaline phosphatase (25 U), and α-lactalbumin (2 mg) were added to 2 mL of 50 mM TRIS buffer, pH 7.4, containing **1** (64 mg, 40 mM), 2 equiv of UDP-glucose (90 mg, 80 mM), and $MnCl_2$ (25 mM). The solution was allowed to react at 30 °C for 5 days, adjusting the pH daily. Reaction outcome was monitored qualitatively by TLC (eluent: AcOEt, MeOH, $H_2O$ 9:2:0.5) and quantitatively by analytical HPLC ($\lambda$: 200 nm; eluent: $H_2O$, $CH_3CN$ 82:18; flow rate: 0.9 mL/min). Purification was performed by preparative HPLC, using the same eluent and injecting the reaction mixture in different portions (flow rate, 5 mL/min), to give 10 mg of **1a** (11% yield), 20 mg of **1b** (26%) and 11 mg of **1c** (13%).

(b) GalT (5 U), epimerase (9 U), alkaline phosphatase (50 U), and α-lactalbumin (10 mg) were dissolved in 1 mL of 50 mM TRIS buffer, pH 7.4, and added to 9 mL of a mixture of the same buffer and DMSO (8:1) containing **1** (160 mg, 20 mM), 5 equivalents of UDP-glucose (610 mg, 100 mM), and $MnCl_2$ (25 mM). The solution was let to react at 30 °C for 24 h, adjusting the pH in the first 8 h and monitoring the conversion by TLC and HPLC. Purification was performed by preparative HPLC, using the same eluent and injecting the reaction mixture in different portions (flow rate, 5 mL/min), to give 72 mg of **1a** (32% yield) and 59 mg of **1b** (31%).

**1a**: amorphous solid; mp 257 °C; $[\alpha]_D$ + 20.8 ($c$ = 0.50, DMSO); HPLC $t_R$ 12.05 min; selected $^1$H NMR data (500.1 MHz, $CD_3OD$−Py-$d_5$) $\delta$ 0.94 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.09 (3H, s), 1.32 (3H, s), 1.34 (3H, s), 1.62 (3H, s), 1.67 (3H, s), 3.10 (1H, dd, $J$ = 11.6, 4.9 Hz, H-3), 3.15 (1H, t, $J$ = 7.8 Hz, H-2‴), 3.27 (1H, t, $J$ = 7.8 Hz, H-2′), 3.36 (1H, m, H-5‴),

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3.40 (1H, m, H-5′), 3.47 (1H, dd, $J$ = 9.8, 3.2 Hz, H-3″), 3.47 (1H, dd, $J$ = 9.8, 3.2 Hz, H-3‴), 3.51 (1H, t, $J$ = 7.8 Hz, H-3′), 3.52 (1H t, $J$ = 7.8 Hz, H-2″), 3.52 (1H, t, $J$ = 7.8 Hz, H-2‴), 3.52 (1H, t, $J$ = 7.8 Hz, H-3‴), 3.54 (1H, m, H-4′), 3.57 (1H, m, H-4‴), 3.57 (1H, m, H-5″), 3.57 (1H, m, H-5‴), 3.68 (1H, td, $J$ = 10.4, 5.3 Hz, H-12), 3.69 (1H, m, H-6a″), 3.69 (1H, m, H-6a‴), 3.76 (1H, m, H-6b″), 3.76 (1H, m, H-6b‴), 3.80 (1H, d, $J$ = 3.2 Hz, H-4″), 3.80 (1H, d, $J$ = 3.2 Hz, H-4‴), 3.80 (1H, m, H-6a‴), 3.80 (1H, m, H-6b‴), 3.82 (1H, m, H-6a′), 3.82 (1H, m, H-6b′), 4.08 (1H, td, $J$ = 10.2 and 3.1 Hz, H-6), 4.35 (1H, d, $J$ = 7.8 Hz, H-1″), 4.35 (1H, d, $J$ = 7.8 Hz, H-1‴), 4.39 (1H, d, $J$ = 7.8 Hz, H-1′), 4.64 (1H, d, $J$ = 7.8 Hz, H-1‴), 5.10 (1H, bt, $J$ = 6.9 Hz, H-24); $^{13}$C NMR (50.3 MHz) $\delta$ 16.11, 17.17, 17.68, 17.83, 17.97, 22.78, 24.22, 25.86, 27.27, 27.61, 30.99, 31.35, 31.59, 36.64, 40.21, 40.40, 40.51, 41.92, 45.39, 49.44, 50.62, 52.44, 53.10, 61.85, 62.40, 62.55 (triple), 70.31 (double), 71.84, 72.62 (double), 74.86 (double), 75.08, 75.15, 76.27, 76.56 (double), 77.08, 77.12, 77.42, 79.89, 80.16, 80.85, 81.00, 85.06, 98.17, 105.05, 105.10, 105.53, 125.82, 132.38; LSIMS $m/z$ 1123 [M − H]$^-$, 961, 799, 637, 475.

**1b**: amorphous solid; mp 211 °C; $[\alpha]_D$ + 24.1 ($c$ = 0.51, DMSO); HPLC $t_R$ 18.83 min; selected $^1$H NMR data (600.0 MHz, $CD_3OD$−Py-$d_5$) $\delta$ 0.73 (3H, s), 0.89 (3H, s), 1.00 (3H, s), 1.15 (3H, s), 1.36 (3H, s), 1.54 (6H, 2s), 1.58 (3H, s), 3.17 (1H, dd, $J$ = 11.6, 4.9 Hz, H-3), 3.40 (1H, t, $J$ = 7.8 Hz, H-2‴), 3.41 (1H, m, H-5‴), 3.53 (1H, m, H-5′), 3.54 (1H, t, $J$ = 7.8 Hz, H-2′), 3.60 (1H, t, $J$ = 7.8 Hz, H-4‴), 3.66 (1H, t, $J$ = 7.8 Hz, H-3‴), 3.67 (1H, td, $J$ = 10.4, 5.3 Hz, H-12), 3.68 (1H, dd, $J$ = 7.8, 3.2 Hz, H-3″), 3.73 (1H, m, H-5″), 3.75 (1H, t, $J$ = 7.8 Hz, H-3′), 3.77 (1H, m, H-4′), 3.83 (1H, dd, $J$ = 12.0, 5.2 Hz, H-6b‴), 3.88 (1H, m, H-6b″), 3.90 (1H, t, $J$ = 7.8 Hz, H-2″), 3.97 (1H, m, H-6a‴), 3.98 (1H, m, H-6a″), 4.01 (1H, d, $J$ = 3.2 Hz, H-4″), 4.03 (1H, m, H-6a′), 4.03 (1H, m, H-6b′), 4.11 (1H, td, $J$ = 10.5, 3.2 Hz, H-6), 4.55 (1H, d, $J$ = 7.8 Hz, H-1′), 4.60 (1H, d, $J$ = 7.8 Hz, H-1″), 4.75 (1H, d, $J$ = 7.8 Hz, H-1‴), 5.10 (1H, bt, $J$ = 6.9 Hz, H-24); $^{13}$C NMR (100.6 MHz) $\delta$ 16.33, 17.27, 17.73, 17.86, 18.05, 22.85, 24.04, 25.98, 27.10, 27.78, 30.94, 31.35, 31.65, 36.50, 40.02, 40.21, 40.56, 41.69, 45.39, 49.41, 50.44, 52.11, 52.77, 61.81, 62.48, 62.59, 62.73, 70.34, 71.36, 71.41, 72.64, 75.11, 75.18, 75.48, 76.37, 77.62, 78.22, 78.67, 79.42, 79.51, 80.73, 81.60, 84.44, 98.51, 105.58, 105.76, 126.05, 131.96; LSIMS $m/z$ 961 [M − H]$^-$, 799, 637, 475.

**1c**: amorphous solid; HPLC $t_R$ 28.19 min; selected $^1$H NMR data (600.0 MHz, $CD_3OD$−Py-$d_5$) $\delta$ 0.72 (3H, s), 0.89 (3H, s), 1.00 (3H, s), 1.19 (3H, s), 1.34 (3H, s), 1.51 (3H, s), 1.54 (3H, s), 1.58 (3H, s), 3.18 (1H, dd, $J$ = 11.6, 4.9 Hz, H-3), 3.45 (1H, t, $J$ = 7.8 Hz, H-2″), 3.48 (1H, m, H-5″), 3.51 (1H, m, H-5′), 3.55 (1H, t, $J$ = 7.8 Hz, H-2′), 3.63 (1H, t, $J$ = 7.8 Hz, H-4′), 3.68 (1H, td, $J$ = 10.4, 5.3 Hz, H-12), 3.70 (1H, t, $J$ = 7.8 Hz, H-3′), 3.72 (1H, dd, $J$ = 7.8, 3.2 Hz, H-3‴), 3.73 (1H, m, H-5‴), 3.78 (1H, t, $J$ = 7.8 Hz, H-3″), 3.78 (1H, t, $J$ = 7.8 Hz, H-4″), 3.88 (1H, m, H-6b′), 3.91 (1H, m, H-6b‴), 3.92 (1H, t, $J$ = 7.8 Hz, H-2‴), 3.98 (1H, m, H-6a‴), 3.99 (1H, m, H-6b″), 4.03 (1H, m, H-6a″), 4.04 (1H, d, $J$ = 3.2 Hz, H-4‴), 4.06 (1H, dd, $J$ = 11.8, 2.5 Hz, H-6a′), 4.13 (1H, td, $J$ = 10.2, 3.1 Hz, H-6), 4.58 (1H, d, $J$ = 7.8 Hz, H-1′), 4.62 (1H, d, $J$ = 7.8 Hz, H-1‴), 4.76 (1H, d, $J$ = 7.8 Hz, H-1″), 5.06 (1H, bt, $J$ = 6.9 Hz, H-24); $^{13}$C NMR (100.6 MHz) $\delta$ 16.43, 17.28, 17.76, 17.90, 18.09, 22.74, 23.95, 26.03, 27.10, 27.83, 30.95, 31.30, 31.71, 31.78, 36.44, 39.99, 40.19, 40.61, 41.67, 45.39, 49.42, 50.44, 52.09, 52.58, 61.78, 62.10, 62.44, 63.10, 70.33, 71.26, 71.92, 72.67, 75.07, 75.16, 75.65, 76.63, 76.98, 77.34, 78.10, 79.51, 80.64, 81.12, 84.49, 98.26, 105.64, 105.97, 126.01, 131.95; LSIMS $m/z$ 961 [M − H]$^-$, 799, 637, 475.

**Ginsenoside F1 (2)** was prepared as described in ref 4: selected $^1$H NMR data (500.1 MHz $CD_3OD$) $\delta$ 0.96 (9H, s), 1.08 (3H, s), 1.28 (3H, s), 1.34 (3H, s), 1.62 (3H, s), 1.67 (3H, s), 3.08 (1H, t, $J$ = 7.8 Hz, H-2′), 3.10 (1H, dd, $J$ = 11.6, 4.9 Hz, H-3), 3.20 (1H, m, H-5′), 3.30 (1H, t, $J$ = 7.8 Hz, H-4′), 3.35 (1H, t, $J$ = 7.8 Hz, H-3′), 3.63 (1H, dd, $J$ = 12.2, 5.2 Hz, H-6a′), 3.67 (1H, td, $J$ = 10.4, 5.3 Hz, H-12), 3.78 (1H, dd, $J$ = 12.2, 2.3 Hz, H-6b′), 4.03 (1H, td, $J$ = 10.2, 3.1 Hz, H-6), 4.60 (1H, d, $J$ = 7.8 Hz, H-1′), 5.10 (1H, bt, $J$ = 6.9 Hz, H-24); $^{13}$C NMR (50.3 MHz) $\delta$ 16.11, 17.22, 17.67 (double), 17.92, 22.80, 24.21, 25.84, 27.19, 27.76, 30.92, 31.45, 31.62, 36.62, 40.14 (double), 40.49, 42.02, 47.21, 49.42, 50.46, 52.36, 53.11, 62.14, 62.57, 68.89, 71.24, 71.80, 75.40, 77.94, 78.27, 79.54, 84.89, 98.30, 125.85, 132.29; LSIMS $m/z$ 637 (M − H)$^-$, 475.

**Enzymatic Galactosylation of Ginsenoside F1.** GalT (5 U), epimerase (15 U), alkaline phosphatase (50 U), and α-lactalbumin (10 mg) were dissolved in 1 mL of 50 mM TRIS buffer, pH 7.4, and added to 9 mL of a mixture of the same buffer and DMSO (7:2) containing **2** (64 mg, 10 mM), 5 equiv of UDP-glucose (305 mg, 50 mM), and $MnCl_2$ (25 mM). The solution was allowed to react at 30 °C for 3 days, adjusting the pH daily. Reaction outcome was monitored qualitatively by TLC (eluent: AcOEt, MeOH, $H_2O$ 9:2:0.5) and quantitatively by analytical HPLC ($\lambda$: 200 nm; eluent: $H_2O$, $CH_3CN$ 72:28; flow rate: 1 mL/min). Purification was performed by preparative HPLC, using the same eluent and injecting the reaction mixture in different portions (flow rate, 6.5 mL/min), to give 41 mg of **2a** (51% yield).

**2a**: amorphous solid; mp 178−180 °C; $[\alpha]_D$ +18.2 ($c$ = 0.33, DMSO); HPLC $t_R$ 26.79 min; selected $^1$H NMR data (500.1 MHz $CD_3OD$) $\delta$ 0.87 (3H, s), 0.88 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.34 (3H, s), 1.39 (3H, s), 1.57 (3H, s), 1.61 (3H, s), 3.14 (1H, dd, $J$ = 11.6, 4.9 Hz, H-3), 3.30 (1H, H-2′), 3.42 (1H, H-5′), 3.60 (1H, dd, $J$ = 7.8, 3.2 Hz, H-3″), 3.66 (1H, t, $J$ = 7.8 Hz, H-3′), 3.67 (1H, td, $J$ = 10.4, 5.3 Hz, H-12), 3.67 (1H, m, H-5″), 3.70 (1H, m, H-4′), 3.73 (1H, t, $J$ = 7.8 Hz, H-2″), 3.80 (1H, dd, $J$ = 11.2, 4.9 Hz, H-6a″), 3.87 (1H, m, H-6b″), 3.89 (1H, m, H-6a′), 3.92 (1H, d, $J$ = 3.2 Hz, H-4″), 3.93 (1H, m, H-6b′), 4.05 (1H, td, $J$ = 10.2, 3.1 Hz, H-6), 4.50 (1H, d, $J$ = 7.8 Hz, H-1″), 4.70 (1H, d, $J$ = 7.8 Hz, H-1′), 5.10 (1H, bt, $J$ = 6.9 Hz, H-24); $^{13}$C NMR (50.3 MHz) $\delta$ 16.32, 17.38, 17.75 (double), 18.05, 22.80, 24.11, 25.97, 27.17, 27.88, 30.92, 31.55, 31.66, 36.54, 40.04 (double), 40.56, 41.91, 47.29, 49.44, 50.87, 52.22, 52.89, 62.01, 62.13, 62.50, 68.70, 70.36, 71.52, 72.69, 75.04 (double), 76.63, 76.81, 77.22, 79.38, 80.63, 84.77, 98.25, 105.36, 125.94, 132.21; LSIMS $m/z$ 799 [M − H]$^-$, 637, 475.

**Ginsenoside Rh$_1$ (3)[18] (20*R* + 20*S*).** Ginsenoside Rg$_1$ (**1**, 1.1 g) was dissolved in 40 mL of 40% v/v AcOH/$H_2O$, and the solution was heated at 70 °C for 1 h and 40 min (TLC, AcOEt, MeOH, $H_2O$ 10:1:0.3). The reaction mixture was diluted with 50 mL of $H_2O$ and extracted with AcOEt. The organic phase was dried, the solvent evaporated, and the crude residue purified by flash chromatography to give 116 mg (14% yield) of **3**. HPLC analysis showed the expected two peaks ($t_R$ = 25.31 and 29.38 min) due to the epimerization of C-20: selected $^1$H NMR data (500.1 MHz, $CD_3OD$) $\delta$ 0.93 (3H, s), 0.99 (3H, s), 1.00 (3H, s), 1.08 and 1.09 and 1.10 and 1.14 (6H, s), 1.32 (3H, s), 1.61 (3H, s), 1.67 and 1.69 (3H, s), 3.09 (1H, m, H-3), 3.18 (1H, t, $J$ = 7.8 Hz, H-2′), 3.33 (1H, t, $J$ = 7.8 Hz, H-3′), 3.25 (1H, m, H-5′), 3.27 (1H, m, H-4′), 3.58 (1H, m, H-12), 3.68 (1H, dd, $J$ = 11.5, 4.9 Hz, H-6a′), 3.80 (1H, dd, $J$ = 12.0, 2.1 Hz, H-6b′), 4.08 (1H, m, H-6), 4.34 and 4.35 (1H, d, $J$ = 7.8 Hz, H-1′), 5.10 and 5.13 (1H, m, H-24); $^{13}$C NMR (50.3 MHz) $\delta$ 16.11, 17.02, 17.29, 17.57, 17.69, 17.84, 22.30, 22.80, 23.28, 25.88, 26.51, 27.21, 27.39, 27.60, 31.39, 31.94, 36.28, 40.20, 40.40, 40.49, 41.87, 43.28, 45.38, 49.85, 50.89, 51.52, 52.52, 52.60, 61.80, 62.93, 71.72, 71.93, 72.07, 74.42, 74.58, 75.51, 77.66, 79.09, 79.87, 80.92, 98.30, 105.56, 125.95, 126.17, 131.97; LSIMS $m/z$ 637 [M − H]$^-$, 475.

**Enzymatic Galactosylation of Ginsenoside Rh$_1$ (3).** Due to its very low solubility, even in the presence of 20% v/v DMSO, galactosylation of **3** could only be monitored by analytical HPLC ($\lambda$: 200 nm; eluent: $H_2O$, $CH_3CN$ 72:28; flow rate: 0.6 mL/min). Reaction mixture: TRIS buffer 50 mM pH 7.4 containing 20% v/v DMSO, 2 mL; **3**, 2.5 mM; UDP-glucose, 25 mM; $MnCl_2$, 25 mM; GalT, 0.2 U; epimerase, 1 U; alkaline phosphatase, 20 U. The only reaction product was recovered by preparative TLC ($R_f$ = 0.34, Rh$_1$ has $R_f$ = 0.48, eluent AcOEt, MeOH, $H_2O$ 2:2:0.5). The product sample was injected into an analytical HPLC column and two peaks were detected at 13.8 and 16.6 min. The LCMS spectrum of each peak showed a [M − H]$^-$ ion at $m/z$ 799.

**6′-*O*-acetyl Rg$_1$ (4)** was prepared as described in ref 4: selected $^1$H NMR data (500.1 MHz $CD_3OD$) $\delta$ 0.94 (3H, s), 0.98 (3H, s), 1.00 (3H, s), 1.09 (3H, s), 1.33 (3H, s), 1.34 (3H, s), 1.61 (3H, s), 1.67 (3H, s), 2.03 (3H, s, $OCOCH_3$), 3.07 (1H, t, $J$

= 7.8 Hz, H-2″), 3.10 (1H, dd, *J* = 11.6, 4.9 Hz, H-3), 3.19 (1H, m, H-5″), 3.19 (1H, t, *J* = 7.8 Hz, H-2′), 3.24 (1H, m, H-4′), 3.29 (1H, m, H-4″), 3.34 (1H, t, *J* = 7.8 Hz, H-3′), 3.35 (1H, t, *J* = 7.8 Hz, H-3″), 3.45 (1H, ddd, *J* = 6.2, 3.4, 2.0 Hz, H-5′), 3.62 (1H, dd, *J* = 12.2, 5.3 Hz, H-6a″), 3.67 (1H, td, *J* = 10.4, 5.3 Hz, H-12), 3.77 (1H, dd, *J* = 12.2, 2.2 Hz, H-6b″), 4.06 (1H, dd, *J* = 11.7, 6.2 Hz, H-6a′), 4.08 (1H, td, *J* = 10.2, 3.1 Hz, H-6), 4.42 (1H, d, *J* = 7.8 Hz, H-1′), 4.47 (1H, dd, *J* = 11.7, 1.8 Hz, H-6b′), 4.60 (1H, d, *J* = 7.8 Hz, H-1″), 5.10 (1H, bt, *J* = 6.9 Hz, H-24); $^{13}$C NMR (50.3 MHz) *δ* 16.28, 17.20, 17.72, 17.81, 17.97, 20.94, 22.84, 24.24, 25.84, 27.12, 27.58, 30.97, 31.31, 31.76, 36.70, 40.22, 40.44 (double), 42.00, 45.66, 49.43, 50.61, 52.41, 53.08, 61.86, 62.59, 65.21, 71.27, 71.55, 71.84, 75.26, 75.42, 75.52, 77.96, 78.29, 78.71, 79.91, 80.40, 84.92, 98.32, 105.50, 125.85, 132.33, 172.60.

**Enzymatic Galactosylation of 6′-*O*-Acetyl Ginsenoside Rg$_1$ (4).** GalT (2.5 U), epimerase (9 U), alkaline phosphatase (25 U), and α-lactalbumin (10 mg) were dissolved in 1 mL of 50 mM TRIS buffer, pH 7.4, and added to 9 mL of a mixture of the same buffer and DMSO (7:2) containing **4** (44 mg, 10 mM), 10 equiv of UDP-glucose (610 mg, 100 mM), and $MnCl_2$ (25 mM). The solution was allowed to react at 30 °C for 3 days, adjusting the pH daily. Reaction outcome was monitored qualitatively by TLC (eluent: AcOEt, MeOH, $H_2O$ 9:2:0.5), while we were not able to find a suitable eluent for a quantitative estimation by analytical HPLC. The reaction mixture was lyophilized and the residue purified by flash chromatography (eluent: AcOEt, MeOH, $H_2O$ 9:3:0.5 to give 36 mg of **4a** (36% yield).

**4a**: amorphous solid; mp 146−147 °C; $[\alpha]_D$ +7.2 (*c* = 0.60, DMSO); $R_f$ = 0.27; selected $^1$H NMR data (500.1 MHz, $CD_3$-OD) *δ* 0.94 (3H, s), 0.98 (3H, s), 1.00 (3H, s), 1.09 (3H, s), 1.33 (3H, s), 1.34 (3H, s), 1.61 (3H, s), 1.67 (3H, s), 2.03 (3H, s, $OCOCH_3$), 3.10 (1H, dd, *J* = 11.6 and 4.9 Hz, H-3), 3.14 (1H, t, *J* = 7.8 Hz, H-2″), 3.20 (1H, t, *J* = 7.8 Hz, H-2′), 3.24 (1H, t, *J* = 7.8 Hz, H-4′), 3.35 (1H, t, *J* = 7.8 Hz, H-3′), 3.36 (1H, m, H-5″), 3.46 (1H, m, H-5′), 3.49 (1H, dd, *J* = 7.8, 3.2 Hz, H-3‴), 3.52 (1H, t, *J* = 7.8 Hz, H-2‴), 3.53 (1H, t, *J* = 7.8 Hz, H-3″), 3.59 (1H, m, H-4″), 3.59 (1H, m, H-5‴), 3.67 (1H, td, *J* = 10.4, 5.3 Hz, H-12) 3.67 (1H, d, *J* = 3.2 Hz, H-4‴), 3.68 (1H, m, H-6a‴), 3.73 (1H, m, H-6b‴), 3.80 (1H, m, H-6a″), 3.83 (1H, m, H-6b″), 4.06 (1H, m, H-6a′), 4.08 (1H, td, *J* = 10.2, 3.1 Hz, H-6), 4.36 (1H, d, *J* = 7.8 Hz, H-1‴), 4.42 (1H, *J* = 7.8 Hz, H-1′), 4.47 (1H, dd, *J* = 11.7, 1.8 Hz, H-6b′), 4.65 (1H, d, *J* = 7.8 Hz, H-1″), 5.09 (1H, bt, *J* = 6.9 Hz, H-24); $^{13}$C NMR (50.3 MHz) *δ* 16.28, 17.22, 17.71, 17.81, 17.98, 20.94, 22.75, 24.20, 25.84, 27.12, 27.58, 30.96, 31.30 (double), 31.77, 36.68, 40.22, 40.43 (double), 41.98, 45.65, 49.42, 50.28, 52.40, 53.00, 61.84, 62.54, 62.74, 65.21, 70.37, 71.55, 71.80, 72.55, 74.77, 75.11, 75.51, 76.42, 76.51, 77.04, 78.65, 79.68, 80.12, 80.35, 85.08, 98.11, 105.06, 105.46, 125.78, 132.39, 172.62; LSIMS *m*/*z* 1003 $[M - H]^-$, 961, 841, 799, 679, 637.

**Ginsenoside Re** (**5**) was isolated by column chromatography from a root extract of Korean Ginseng supplied by Indena, Milano (Italy): selected $^1$H NMR data (500.1 MHz, $CD_3OD$) *δ* 0.94 (3H, s), 0.95 (3H, s), 0.96 (3H, s), 1.04 (3H, s), 1.22 (3H, d, *J* = 6.1 Hz, H-6″), 1.33 (3H, s), 1.34 (3H, s), 1.61 (3H, s), 1.67 (3H, s), 3.08 (1H, t, *J* = 7.8 Hz, H-2‴), 3.13 (1H, dd, *J* = 11.6, 4.9 Hz, H-3), 3.21 (1H, m, H-5‴), 3.30 (1H, m, H-5′), 3.30 (1H, m, H-4‴), 3.35 (1H, m, H-4′), 3.35 (1H, t, *J* = 7.8 Hz, H-3‴), 3.38 (1H, m, H-4″), 3.48 (1H, t, *J* = 7.8 Hz, H-2′), 3.52 (1H, t, *J* = 7.8 Hz, H-3′), 3.62 (1H, dd, *J* = 12.2, 2.3 Hz, H-6a‴), 3.66 (1H, td, *J* = 10.4, 5.3 Hz, H-12), 3.68 (1H, m, H-3″), 3.69 (1H, m, H-6a′), 3.76 (1H, dd, *J* = 12.2, 2.2 Hz, H-6b‴), 3.86 (1H, dd, *J* = 12.2, 2.3 Hz, H-6b′), 3.89 (1H, dd, *J* = 3.2, 1.4 Hz, H-2″), 4.08 (1H, m, H-5″), 4.35 (1H, td, *J* = 10.2, 3.1 Hz, H-6), 4.59 (1H, d, *J* = 7.8 Hz, H-1‴), 4.64 (1H, d, *J* = 7.8 Hz, H-1′), 5.10 (1H, bt, *J* = 6.9 Hz, H-24), 5.31 (1H, d, *J* = 1.4 Hz, H-1″); $^{13}$C NMR (50.3 MHz) *δ* 17.22, 17.32, 17.47, 17.69, 17.94, 18.04, 22.84, 24.24, 25.86, 27.29, 27.55, 30.96, 31.76, 31.97, 36.65, 40.26 (double), 40.38, 41.99, 46.07, 49.43, 50.28, 52.48, 53.13, 61.46, 62.57, 63.12, 69.67, 71.25., 71.91 (double), 72.24, 72.47, 74.04, 74.95, 75.42, 77.95, 78.09, 78.27, 79.16 (double), 79.80, 84.94, 98.33, 101.62 (double), 125.85, 132.32; LSIMS *m*/*z* 945 $[M - H]^-$, 783, 637.

**Enzymatic Galactosylation of Ginsenoside Re (5).** GalT (3.5 U), epimerase (9 U), alkaline phosphatase (25 U), and α-lactalbumin (10 mg) were dissolved in 1 mL of 50 mM TRIS buffer, pH 7.4, and added to 9 mL of a mixture of the same buffer and DMSO (7:2) containing **5** (50 mg, 5 mM), 5 equiv of UDP-glucose (152 mg, 25 mM), and $MnCl_2$ (25 mM). The solution was allowed to react at 30 °C for 2 days, adjusting the pH. Reaction outcome was monitored qualitatively by TLC (eluent: AcOEt, MeOH, $H_2O$ 10:4:0.5), while we were not able to find a suitable eluent for a quantitative estimation by analytical HPLC. The reaction mixture was lyophilized and the residue purified by flash chromatography (eluent: AcOEt, MeOH, $H_2O$ 10:4:0.5) to give 24 mg of **5a** (41% yield).

**5a**: amorphous solid; mp 210−212 °C; $[\alpha]_D$ +0.6 (*c* = 0.33, DMSO); $R_f$ = 0.08; selected $^1$H NMR data (500.1 MHz, $CD_3$-OD) *δ* 0.94 (3H, s), 0.95 (3H, s), 0.96 (3H, s), 1.09 (3H, s), 1.22 (3H, d, *J* = 6.1 Hz, H-6″), 1.33 (3H, s), 1.34 (3H, s), 1.62 (3H, s), 1.68 (3H, s), 3.13 (1H, dd, *J* = 11.6, 4.9 Hz, H-3), 3.14 (1H, t, *J* = 7.8 Hz, H-2‴), 3.30 (1H, m, H-5′), 3.35 (1H, m, H-4′), 3.36 (1H, m, H-5‴), 3.37 (1H, m, H-4″), 3.37 (1H, m, H-5⁗), 3.47 (1H, m, H-3⁗), 3.49 (1H, t, *J* = 7.8 Hz, H-2′), 3.52 (1H, t, *J* = 7.8 Hz, H-3′), 3.53 (1H, t, *J* = 7.8 Hz, H-3‴), 3.53 (1H, t, *J* = 7.8 Hz, H-2⁗), 3.58 (1H, m, H-4‴), 3.65 (1H, td, *J* = 10.4 and 5.3 Hz, H-12), 3.68 (1H, m, H-3″), 3.68 (1H, m, H-6a⁗), 3.69 (1H, m, H-6a′), 3.76 (1H, dd, *J* = 10.4, 7.6 Hz, H-6b⁗), 3.79 (1H, m, H-6a‴), 3.81 (1H, m, H-6b‴), 3.81 (1H, d, *J* = 3.2 Hz, H-4⁗), 3.86 (1H, dd, *J* = 11.8, 2.5 Hz, H-6b′), 3.89 (1H, dd, *J* = 3.2, 1.4 Hz, H-2″), 4.08 (1H, m, H-5″), 4.34 (1H, td, *J* = 10.2, 3.1 Hz, H-6), 4.35 (1H, d, *J* = 7.8 Hz, H-1⁗), 4.64 (1H, d, *J* = 7.8 Hz, H-1′), 4.64 (1H, d, *J* = 7.8 Hz, H-1‴), 5.10 (1H, bt, *J* = 6.9 Hz, H-24), 5.31 (1H, d, *J* = 1.4 Hz, H-1″); $^{13}$C NMR (50.3 MHz) *δ* 17.23, 17.32, 17.47, 17.69, 17.96, 18.03, 22.77, 24.22, 25.86, 27.31, 27.54, 30.96, 31.77, 31.97, 36.64, 40.26, 40.38 (double), 41.99, 46.06, 49.43, 50.28, 52.48, 53.10, 61.45, 61.86, 62.51, 63.12, 69.67, 70.33, 71.91 (double), 72.24, 72.47, 72.62, 74.04, 74.85 (double), 75.08, 76.55, 77.07, 78.09 (double), 79.16 (double), 79.79, 80.11, 85.05, 98.18, 101.63 (double), 105.04, 125.80, 132.39; LSIMS *m*/*z* 1107 $[M - H]^-$, 961, 945, 799, 783, 637.

**Enzymatic Glucosylation of Ginsenoside Rg$_1$.** GalT (5 U), alkaline phosphatase (25 U), and α-lactalbumin (10 mg) were dissolved in 1 mL of 50 mM TRIS buffer, pH 7.4, and added to 9 mL of a mixture of the same buffer and DMSO (7:2) containing **1** (160 mg, 20 mM), 5 equivalents of UDP-glucose (610 mg, 100 mM), and $MnCl_2$ (25 mM). The solution was let to react at 30 °C for 5 days, adjusting the pH daily. Reaction outcome was monitored qualitatively by TLC (eluent: AcOEt, MeOH, $H_2O$ 9:2:0.5) and quantitatively by analytical HPLC (*λ*: 200 nm; eluent: $H_2O$, $CH_3CN$ 82:18; flow rate: 1.0 mL/min). The conversion after 4 days was 42.1%. Purification was performed by preparative HPLC, using the same eluent and injecting the reaction mixture in different portions (flow rate, 7.5 mL/min), to give 13 mg of **1d** (6% yield) and 46 mg of **1e** (24% yield).

**1d:** amorphous solid; mp 224−226 °C; $[\alpha]_D$ +32.0 (*c* = 0.1, DMSO); HPLC $t_R$ 13.84 min; selected $^1$H NMR data (500.1 MHz, $CD_3OD$) *δ* 0.95 (3H, s), 0.98 (3H, s), 0.99 (3H, s), 1.09 (3H, s), 1.32 (3H, s), 1.34 (3H, s), 1.62 (3H, s), 1.67 (3H, s), 3.08 (1H, t, *J* = 7.8 Hz, H-2‴), 3.10 (1H, dd, *J* = 11.6, 4.9 Hz, H-3), 3.19 (1H, m, H-5‴), 3.21 (1H, t, *J* = 7.8 Hz, H-2″), 3.26 (1H, t, *J* = 7.8 Hz, H-2′), 3.29 (1H, m, H-4‴), 3.31 (1H, m, H-4″), 3.34 (1H, m, H-5″), 3.34 (1H, m, H-3‴), 3.35 (1H, m, H-3″), 3.39 (1H, m, H-5′), 3.49 (1H, m, H-3′), 3.54 (1H, m, H-4′), 3.62 (1H, m, H-6a‴), 3.65 (1H, m, H-6a″), 3.67 (1H, m, H-12), 3.77 (1H, dd, *J* = 12.0, 2.3 Hz, H-6b‴), 3.82 (2H, m, H-6a′ and H-6b′), 3.87 (1H, dd, *J* = 12.0, 2.3 Hz, H-6b″), 4.08 (1H, td, *J* = 10.2, 3.1 Hz, H-6), 4.38 (1H, d, *J* = 7.8 Hz, H-1′), 4.40 (1H, d, *J* = 7.8 Hz, H-1″), 4.59 (1H, d, *J* = 7.8 Hz, H-1‴), 5.10 (1H, bt, *J* = 6.9 Hz, H-24); $^{13}$C NMR (50.3 MHz) *δ* 16.09, 17.13, 17.66, 17.80, 17.93, 22.81, 24.81, 25.84, 27.21, 27.58, 30.95, 31.34, 31.56, 36.64, 40.18, 40.39, 40.48, 41.90, 45.37, 49.42, 50.60, 52.42, 53.12, 61.83, 62.49, 62.70, 63.10, 70.92, 71.42, 71.86, 73.53, 74.97, 75.14, 75.40, 76.25, 77.39, 77.92, 78.12, 78.27, 79.87, 79.99, 80.97, 84.92, 98.30, 104.59, 105.49, 125.86, 132.29; LSIMS *m*/*z* 961 $[M - H]^-$, 799, 637, 475.

**1e:** amorphous solid; mp 218−220 °C; $[\alpha]_D$ + 17.6 ($c$ = 0.17, DMSO); HPLC $t_R$ 32.76 min; selected $^1$H NMR data (399.9 MHz, $CD_3OD$) $\delta$ 0.94 (3H, s), 0.98 (3H, s), 1.00 (3H, s), 1.09 (3H, s), 1.32 (3H, s), 1.33 (3H, s), 1.62 (3H, s), 1.67 (3H, s), 3.10 (1H, m, H-3), 3.14 (1H, m, H-2″), 3.19 (1H, m, H-2′), 3.19 (1H, m, H-2‴), 3.28 (1H, m, H-4′), 3.32 (1H, m, H-4‴), 3.34 (1H, m, H-5″), 3.34 (1H, m, H-3‴), 3.57 (1H, m, H-4″), 3.58 (1H, m, H-6a′), 3.65 (1H, m, H-6a‴), 3.67 (1H, m, H-12), 3.78 (1H, m, H-6b′), 3.80 (2H, m, H-6a″ and H-6b″), 3.88 (1H, m, 6b‴), 4.08 (1H, td, $J$ = 10.2, 3.1 Hz, H-6), 4.35 (1H, d, $J$ = 7.7 Hz, H-1‴), 4.41 (1H, d, $J$ = 7.9 Hz, H-1′), 4.64 (1H, d, $J$ = 7.9 Hz, H-1″), 5.10 (1H, bt, $J$ = 6.9 Hz, H-24); $^{13}$C NMR (100.6 MHz) $\delta$ 16.10, 17.11, 17.64, 17.83, 17.96, 22.74, 24.22, 25.86, 27.26, 27.59, 30.95, 31.38, 31.55, 36.63, 40.17, 40.37, 40.50, 41.88, 45.29, 49.42, 50.61, 52.45, 53.09, 61.78, 62.43, 62.92, 63.10, 70.93, 71.38, 71.73, 71.84, 74.95, 75.16, 75.51, 76.56, 77.69, 77.86, 78.10, 79.10, 79.85, 80.04, 80.93, 85.05, 98.13, 104.51, 105.58, 125.80, 132.37; LSIMS $m/z$ 961 $[M - H]^-$, 799, 637, 475.

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**Supporting Information Available:** Spectrometric information ($^1$H and $^{13}$C NMR) for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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