Regioselective Enzyme-Mediated Acylation of Polyhydroxy Natural Compounds. A Remarkable, Highly Efficient Preparation of 6′-*O*-Acetyl and 6′-*O*-Carboxyacetyl Ginsenoside Rg_1

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Lipase B from *Candida antarctica* has been shown to be an efficient catalyst for the regioselective acylation of the dammarane type glucoside ginsenoside Rg_1 (**1**) on reaction with vinyl acetate in *t*-AmOH, affording the corresponding 6′-*O*-acetyl derivative **1b** in high yield. The structure of **1b** was determined through a careful inspection of its ^1H NMR at 600 MHz, which allowed for the complete assignment of the signals of the sugar's protons. The introduction of a carboxyacetyl residue was then investigated using different protocols. The best results were obtained with a two-step sequence involving the preliminary enzymatic acylation of **1** with bis(2,2,2-trichloroethyl) malonate to give the mixed malonyl derivative **1f**, followed by selective chemical hydrolysis with Zn/AcOH to the 6′-*O*-carboxyacetyl ginsenoside Rg_1 (**1e**).

Introduction

Ginsenosides are therapeutic dammarane type triterpene oligoglycosides isolated from the water soluble portion of the dried roots and leaves of *Panax* ginseng C. A. Meyer (*Araliaceae*), a plant widely used in traditional Chinese medicine.[1] Careful examination of white ginseng[2] extract has revealed that some ginsenosides are present as monoesters of malonic acid, acylation occurring invariably at one of the primary OH's of the sugar moieties.[3] These carboxyacetyl ginsenosides behave as acidic saponins, and besides being more soluble in water than the ordinary glycosides, they also cause a remarkable increase in the water solubility of the other ginsenosides. In spite of these interesting properties, which can deeply influence the absorption of these drugs in humans, to our knowledge, no information is available on the synthesis of malonyl ginsenosides or, more generally, on the synthesis of specific esters of ginsenosides with aliphatic carboxylic acids.

Recently, we have successfully exploited the ability of the proteolytic enzyme subtilisin (protease Carlsberg) to catalyze the regioselective acylation of different flavonoid monoglucosides and disaccharide monoglycosides with activated esters in anhydrous polar organic solvents.[4,5] This methodology seemed very attractive for the regioselective esterification of other more complex glycosides, and, to broaden its application, we started a research program examining the behavior of some ginsenosides. The aim was to obtain new derivatives having modified solubility properties with respect to the ordinary ginsenosides and, eventually, to have a synthetic access to the carboxyacetyl ginsenosides. We report here on the results obtained for the acylation of ginsenoside Rg_1 (**1**, Rg_1, 6,20-di-*O*-glucopyranosyl-20(*S*)-protopanaxatriol), one of the simplest and most abundant glycosides isolated from *P. ginseng*.[6]

Results and Discussion

Following the protocol for the esterification of flavonoid glycosides,[4] we first attempted to use the protease subtilisn to acylate our target ginsenoside Rg_1 (**1**), but a

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1995.

(1) For a comprehensive review on the isolation, structure elucidation, chemistry, and pharmacology of these compounds, see: Tanaka, O.; Kasai, R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Griesbach, H., Kirby, G. W., Tamm, C. H., Eds.; Springer-Verlag: Vienna, 1984; pp 1–76.

(2) Ginseng is often processed in two forms, white and red ginseng; white ginseng is the dried root, while the red one is the steamed root, which shows a caramel-like color: Steinhegger, E. *Lehrbuch der Pharmacognosie und Phytopharmazie*, 4th ed.; Springer-Verlag: Berlin, 1988; pp 615–628.

(3) Kitagawa, I.; Taniyama, T.; Yoshikawa, M.; Ikenishi, Y.; Nagakawa, Y. *Chem. Pharm. Bull.* **1989**, *37*, 2961.

(4) (a) Danieli, B.; De Bellis, P.; Carrea, G.; Riva, S. *Heterocycles* **1989**, *29*, 2061. (b) Danieli, B.; De Bellis, P.; Carrea, G.; Riva, S. *Helv. Chim. Acta* **1990**, *73*, 1837.

(5) The regioselective acylation of glycosides by chemical reagents represents a challenging problem due to the multiple hydroxyls present in the molecule, and a clear discrimination among primary and secondary hydroxyls and especially among primary OH's of various saccharides of the same molecule still remains a difficult task: (a) Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 11. (b) Haines, A. H. *Ibid.* **1981**, *39*, 13. (c) Kahn, R. *Pure Appl. Chem.* **1984**, *56*, 883. (d) Kowacs, P.; Sokoloski, E. A.; Glaudemans, C. P. *J. Carbohydr. Chem.* **1984**, *101*, 128.

(6) (a) Iida, Y.; Tanaka, O.; Shibata, S. *Tetrahedron Lett.* **1968**, 5449. (b) Nagay, Y.; Tanaka, O.; Shibata, S. *Tetrahedron* **1971**, *27*, 881.

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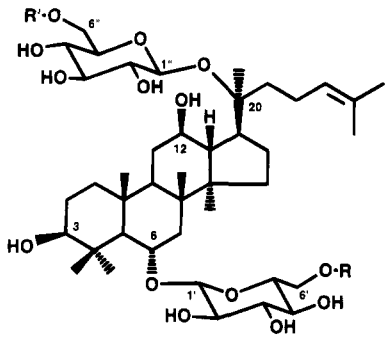
slow nonselective conversion (about 30%) to a mixture of at least three main acylated components occurred.[7] A



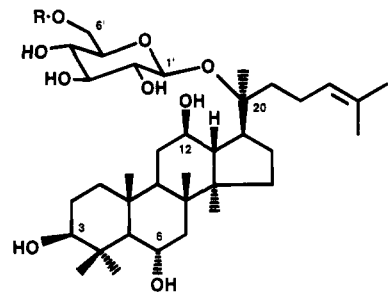
1 R = R' = H
1a R = R' = Ac
1b R = Ac ; R' = H
1c R = $COCH_2COOCH_2CF_3$; R' = H
1d R = $COCH_2COOCH_3$; R' = H
1e R = $COCH_2COOH$; R' = H
1f R = $COOCH_2COOCH_2CCl_3$; R' = H

study was therefore undertaken to find more suitable conditions (enzyme source and nature of solvent and acylating agent) for obtaining the desired selective transformation.

After experimentation, the most satisfactory results were obtained with the now commercially available immobilized *Candida antarctica* lipase B (*C. a.* B lipase)[8] in *tert*-amyl alcohol as solvent and vinyl acetate as acylating agent. Under these conditions, a clean and complete conversion of **1** to only two products in a 22:1 ratio was obtained after 6 h.

The less abundant and less polar product was identified as 6′,6″-di-*O*-acetyl Rg_1 (**1a**) on the basis of its FAB mass spectrum [$(M + Na)^+$ at *m/z* 907, $(M + H)^+$ at *m/z* 885] and its ^1H NMR spectrum, which contained two singlets at 2.00 and 1.99 ppm (CH_3COO) and two downfield-shifted AB portions of two ABX systems at 4.37 and 4.31 ppm and at 4.01 and 3.96 ppm, respectively (the acylated CH_2-6′ and CH_2-6″ of the two glucose moieties). In the ^{13}C NMR spectrum, the primary oxymethylene carbons appeared at 63.8 and 64.1 ppm compared to 61.69 ppm (two superimposed signals) for the starting material.

The most abundant product, **1b**, was a monoacetyl derivative displaying a $(M + H)^+$ at *m/z* 843 in the FAB spectrum. Two intense fragment ions were sequentially formed and detected at *m/z* 645 (loss of glucose and H_2O) and 422 (loss of acetylated glucose) by B/E-linked scan fragmentation analysis. These data, in agreement with the well-known mass spectrometer-induced preferential elimination of oxygenated function at C-20 in ginsenoside derivatives,[9] were taken as strong evidence for acetylation on the glucose linked at C-6 OH, probably at its primary OH. Further support for this hypothesis was gained by comparison of the enzymatic esterification of Rg_1 with its more simple congener ginsenoside F_1 (**2**, F_1) lacking the glucose moiety at C-6 OH.[10] In fact, a parallel study showed that, while **1** was completely transformed in about 6 h, **2** was poorly acetylated (about 28%), even after 48 h, at the primary OH of its C-20 glucose moiety to give **2a**.



2 R = H
2a R = Ac

For an unequivocal structural assignment of the monoacetylated Rg_1 (**1b**), a detailed NMR study was then undertaken. In spite of the pharmacological importance of ginsenosides and the extensive chemical studies on their intriguing structures, very little ^{13}C NMR data are available.[1,10b,11] In the case of Rg_1, only the signals of the genine carbons and the two anomeric carbons C-1′ and C-1″ were assigned, whereas the attribution of the closely spaced signals due to C-2, C-3, C-4, C-5, and C-6 of the two glucose moieties was never attempted. For the ^1H NMR, only scant data are currently reported[1,3] due to extensive signal superimposition, relevant to H-24, to the (not assigned) anomeric protons and to the genine methyls.

In order to unambiguously locate the position of the acetyl group, we have attempted a complete assignment of proton signals by exploiting the inherent resolving power of a high-field NMR instrument (600 MHz) and by using modern 2D-correlation pulse sequences. The ^1H NMR spectrum of **1b** in DMSO is reported in Figure 1, while the spectral data are collected in Table 1.

Total correlation spectroscopy (TOCSY)[12] experiments allowed us to locate the high-field anomeric proton at 4.29 ppm on the same glucose moiety as the low-field protons of the acetylated oxymethylene at 4.32 and 3.91 ppm. In addition, the low-field anomeric proton was, in turn, related to the protons of the unsubstituted oxymethylene at 3.62 and 3.40 ppm.

A homonuclear DQF-COSY experiment made the assignment of the other protons to the corresponding position within the monosaccharide unit straightforward. Combining TOCSY and COSY information, we could also assign the great majority of protons of the dammarane skeleton. In particular, the structurally important H-17 and H-7_{eq} were located at 2.18 and at 1.88 ppm, respectively.

(7) The lack of selectivity displayed by subtilisin against Rg_1 paralleled its behavior toward other triterpenoids glucosides. For example, we have shown that sitosterol-3′-β-*O*-glucopyranoside furnished complex mixtures of mono- and diacylated products under similar reaction conditions.

(8) Other lipases (e.g. from *Chromobacterium viscosum*, *Mucor miehei*, *Pseudomonas cepacea*, and pig pancreas) gave unsatisfactory and nonselective conversion.

(9) Fraisse, D.; Tabet, J. C.; Becchi, M.; Raynaud, J. *Biomed. Mass Spectrom.* **1986**, *13*, 1.

(10) (a) Yahara, S.; Tanaka, O.; Komori, T. *Chem. Pharm. Bull.* **1976**, *24*, 2204. (b) Yahara, S.; Kaji, K.; Tanaka, O. *Chem. Pharm. Bull.* **1979**, *27*, 88.

(11) (a) Kasai, R.; Susuo, M.; Asakawa, J.; Tanaka, O. *Tetrahedron Lett.* **1977**, 175. (b) Asakawa, J.; Kasai, R.; Yamasaki, K.; Tanaka, O. *Tetrahedron* **1977**, *33*, 1935.

(12) (a) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521. (b) Bax, A.; Griffey, R. H. *J. Magn. Reson.* **1985**, *65*, 355.

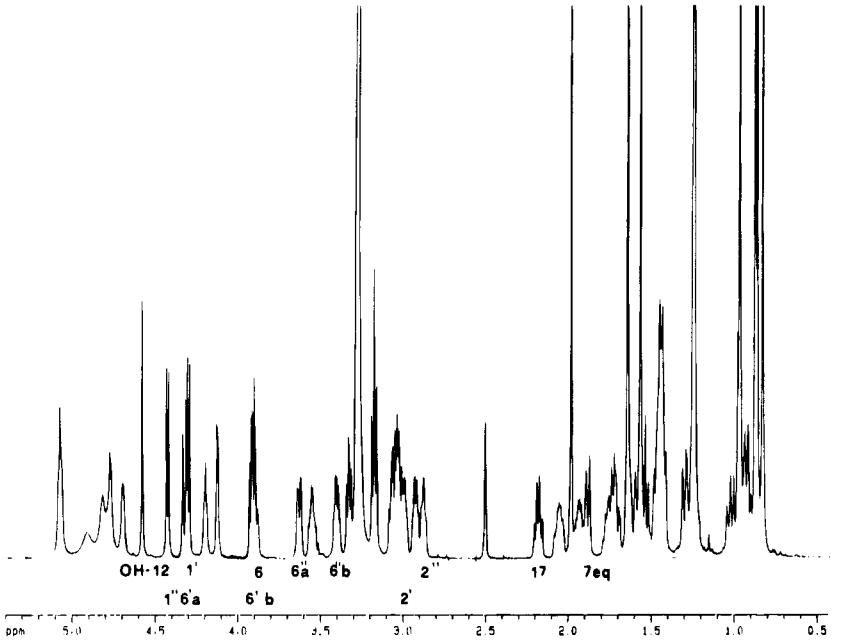


Figure 1. ^{1}H-NMR spectrum (600 MHz) of **1b** in DMSO.

In order to assign each monosaccharide spin system either to the C-6 OH glucose (glucose') or to the C-20 glucose (glucose"), 2D-NOE experiments (NOESY) were performed. We observed that the above-mentioned H-17 had a strong through-space interaction with the anomeric signal at 4.42 ppm, which was therefore assigned to H-1", and that C-12 OH had a contact with H-2" at 2.87 ppm. Moreover, H-6 and H-7_{eq} showed a connectivity with the anomeric proton at 4.29 ppm, therefore identified as H-1'.

These interactions were useful in defining the mutual orientation of the two glucoses with respect to the tetracyclic skeleton. The NOE contacts were used to drive the energy minimization of a molecular model, by means of restrained molecular mechanics calculations.[13] The conformation depicted in Figure 2 was obtained with a subsequent unrestrained energy minimization and completely conformed to experimental data.

The ^{13}C NMR spectrum was measured in DMSO at 35 °C. The assignments are based on ^{1}H−^{13}C COSY experiments *via* one-bond couplings in the reverse detection mode (HMQC)[14] and on the previous proton assignments. Even the few proton signals of the dammarane skeleton left unassigned in the homonuclear experiments were attributed, taking advantage of the chemical shift dispersion in the ^{13}C frequency domain (Table 1).

From all these data, the structure of 6'-*O*-acetyl ginsenoside Rg_1 (**1b**) has been completely substantiated.

We next investigated the introduction of a carboxyacetyl residue into the Rg_1 molecule. With the activated ester methyl 2,2,2-trifluoroethyl malonate, Rg_1 underwent a complete transformation into a 1:2 mixture of two products, the 6'-*O*-((2,2,2-trifluoroethoxy)carbonyl)acetyl ginsenoside Rg_1 (**1c**)[15] and the anticipated 6'-(*O*-methoxycarbonyl)acetyl ginsenoside Rg_1 (**1d**). Therefore, in order to obtain a single compound, **1d** was subsequently prepared in 86% isolated yield, employing dimethyl malonate as acylating agent.

6'-(*O*-Methoxycarbonyl)acetyl ginsenoside Rg_1 (**1d**) was then subjected to selective enzymatic hydrolysis of the methoxycarbonyl function by action of another enzyme, the pig liver esterase.[16] The reaction proceeded with an acceptable degree of selectivity, affording the desired 6'-*O*-carboxyacetyl ginsenoside Rg_1 (**1e**) together with a small amount of the starting ginsenoside Rg_1 (**1**) (as indicated by TLC). However, **1e** could be isolated only in small amounts by flash chromatography, due to its propensity to undergo complete hydrolysis. Its negative FABMS spectrum showed the expected $(M-H)^-$ ion at *m/z* 885, accompanied by an intense fragment ion at *m/z* 841, caused by the loss of CO_2. Compared to **1c**, the ^{13}C NMR spectrum of **1e** was lacking the signal at 52.20 ppm due to the methyl group of the methoxycarbonyl function. The ^{1}H NMR spectrum, recorded in DMSO-d_6 at room temperature, displayed rather broad signals, which prevented a detailed interpretation. On the NMR tube being heated at 80 °C, a rapid decarboxylation of **1e** occurred, and a spectrum identical to that of 6'-*O*-acetyl ginsenoside Rg_1 (**1b**) was obtained.

In an attempt to improve the yield of **1e**, we tested an alternative chemoenzymatic approach based on the enzymatic formation of 6'-*O*-((2,2,2-trichloroethoxy)carbonyl)acetyl ginsenoside Rg_1 (**1f**), followed by the chemical removal of 2,2,2-trichloroethanol by action of Zn/AcOH.[17] Acylation of **1** with bis(2,2,2-trichloroethyl) malonate and

(13) Molecular modeling was performed on a Silicon Graphics 4D35GT workstation equipped with Insight/Discover software (v. 2.2.0, Biosym Technologies, San Diego). A molecular model was built from fragments present in the software library, with standard bond lengths and angles. It was energy-minimized using conjugated gradients until the maximum energy derivatives were less than 0.1 kcal/mol^{-1} A^{-1}. NOE interactions were used as an upper bound constraint of 3 A.

(14) Bax, A.; Griffey, R. H.; Hawkins, B. L. *J. Magn. Reson.* **1983**, *55*, 301.

(15) The formation of **1c** was rather surprising because the (2,2,2-trifluoroethoxy)carbonyl function of methyl 2,2,2-trifluoroethyl malonate was expected to be much more reactive than the methoxycarbonyl one in forming the acyl enzyme intermediate. See ref 16.

(16) For the use of pig liver esterase in a similar hydrolysis, see: Danieli, B.; Bertario, A.; Carrea, G.; Redigolo, B.; Secundo, F.; Riva, S. *Helv. Chim. Acta* **1993**, *76*, 2981.

(17) Fabre, J.; Betbeder, D.; Paul, F.; Monsan, P.; Perir, J. *Tetrahedron* **1993**, *49*, 10877.

Table 1. ^1H and ^{13}C NMR Data of 6′-*O*-Acetyl Ginsenoside Rg_1 (1b)

H or C	H (ppm)[a]	multiplicity	*J* (Hz)	NOE to protons[b]	*C* (ppm)[c]
1	0.93(ax)	m			38.80
	1.58	dt	13, 3		
2	1.4−1.5	m			26.68
3	2.92	dt	11.5, 5.5	$H\text{-}1_{ax}$, H-5; CH_3-28	77.07
4					38.70[e]
5	0.97	d	11		60.12
6	3.89	td	11, 3.5	$H\text{-}7_{eq}$; CH_3-18, 19, 29; H-1′	78.23
7	1.43	m			44.12
	1.88(eq)	dd	13, 3.5	H-1′; H-6	
8					40.09[e]
9	1.30	dd	13, 3		48.72
10					38.43[e]
11	1.01	m			30.50
	1.63	m			
12	3.54	tdd	11, 5.5, 1.5	H-17; H-9; CH_3-30; 12-OH	68.85
13	1.53	t	11		48.08
14					50.31
15	0.91	m			30.02
	1.43	m			
16	1.24	m			25.51
	1.78	m			
17	2.18	td	11, 9	*H-1′*; 12-OH; H-12; CH_2-16	50.48
18	0.96	s			16.75
19	0.87	s			16.84
20					81.94
21	1.25	s		H-1″	21.47
22	1.44	m			35.28
	1.71	m			
23	1.93	m			22.10
	2.05	m			
24	5.07	br t	7		125.22
25					130.10
26	1.56	br s			17.23
27	1.64	br s			25.32
28	1.24	s		H-3	29.95
29	0.86	s		H-6	15.47
30	0.83	s		H-12; H-17	16.80
1′	4.29	d	7.8	H-3′; H-5′; *H-6*; $H\text{-}7_{eq}$	104.45
2′	2.99	ddd	9, 7.8, 4.5		73.76[e]
3′	3.18	t	9		77.15
4′	3.02	dd	9, 8.5		69.97[e]
5′	3.32	ddd	8.5, 7, 2		73.81[e]
6′	4.32	dd	11.5, 2		64.07
	3.91	dd	11.5, 7		
1″	4.42	d	7.8	*H-17*; H-3″; H-5″; *CH_3-21*	96.55
2″	2.87	td	9, 7.8, 4.5	*12-OH*; H-4″	73.49
3″	3.18	t	9		77.15
4″	3.03	dd	10, 9		70.11[e]
5″	3.07	ddd	10, 6, 2		76.49
6″	3.63	ddd	11.5, 6, 2		61.13
	3.40	ddd	11.5, 6, 6		
CH_3COO	1.98	s			20.52
CH_3COO					170.05
OH-3 4.13; OH-12 4.58;[d] OH-2′ 4.70; OH-4′ 5.07; OH-2″ 4.77; OH-4″ 4.82; OH-6″ 4.20					

[a] At 600 MHz, DMSO-d_6, 35 °C. [b] Only structural relevant connectivities are reported (strong interactions in italics). [c] At 150 MHz, DMSO-d_6 + D_2O, 35 °C. [d] NOE connectivities to H-2′, H-12, H-13, and H-16 at 1.78, H-17, H_2-11, and CH_3-30. [e] Assignments might be exchanged.

C. a. B. lipase gave **1f** in good yield.[18] Hydrolysis of **1f** by action of Zn/AcOH afforded the carboxyacetyl derivative **1e**, identical to the previously isolated compound. Both the enzymatic and chemical reactions of this sequence were quite clean; however, in this case too, the final chromatographic purification of **1e** was somewhat troublesome, the product always being contaminated by a variable amount of the parent ginsenoside Rg_1. Not surprisingly, **1e** has never been found in ginseng extracts.[3] Probably the isolation of this compound is greatly hampered by its observed instability, as reported process conditions for the preparation of the extracts[6,10] require extraction of roots with hot MeOH and extensive chromatographic purifications.

In conclusion, we have shown that *C. a.* B lipase is a very efficient catalyst for the regioselective esterification of the polyhydroxylated compound ginsenoside Rg_1. In the frame of this work, an extensive ^1H NMR study of 6′-*O*-acetyl ginsenoside Rg_1 has been performed at 600 MHz, allowing for the first time for the complete assignment of the signals due to the saccharides and genine moieties.

Work is in progress to study the behavior of this enzyme toward even more complex and bulkier ginseno-

(18) Compound **1f** proved to be very unstable in the presence of nucleophiles. For instance, by standing in a methanolic solution for a few hours at room temperature, **1f** was completely converted into **1c**. Also, the FABMS spectrum showed peaks derived by and extensive interaction with the matrix (see Experimental Section).

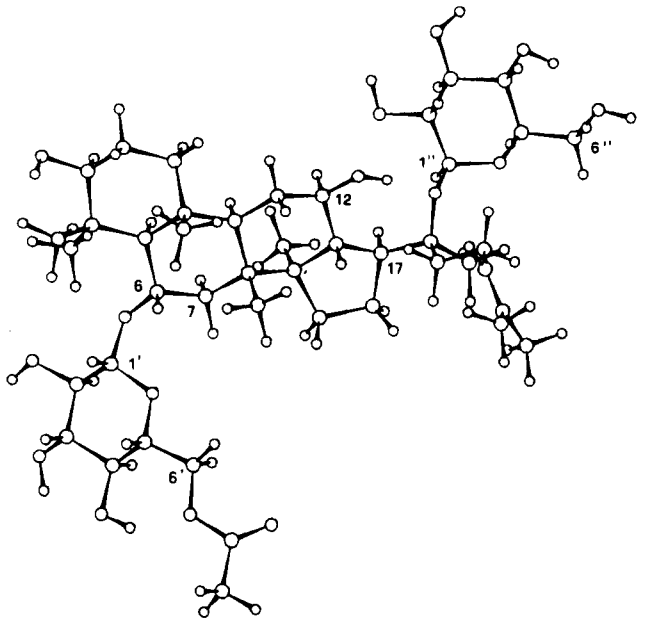


Figure 2. Energy-minimized structure of **1b**.

sides like, for instance, the disaccharide diglucoside ginsenoside Rb_1.[1,19]

Experimental Section

Materials and Methods. Hesperidinase, pig liver esterase, and pig pancreatic lipase were purchased from Sigma. Lipase B from *C. antarctica* (*C. a.* B lipase), immobilized on an acrylic resin (Novozym 435), was a generous gift from Novo-Nordisk. Lipase from *Pseudomonas cepacia* was from Amano, lipase from *Chromobacterium viscosum* was from Finnsugar, while lipase from *Mucor miehei* was from Biocatalysts. Enzymatic transesterifications of **1** were followed by TLC with precoated silica gel 60 F_{254} plates (Merck). Compounds were detected with the Komarowsky's reagent.[20]

Melting points were determined on a Kofler melting point microscope.

^1H and ^{13}C NMR spectra at 300 MHz and 75.2 MHz were recorded in DMSO-d_6 + D_2O at 80 °C. NMR spectra at 600 MHz (^1H, homonuclear DQF-COSY, TOCSY, ^1H–^{13}C COSY, and 2D-NOESY) and at 150 MHz (^{13}C) were run in DMSO-d_6 at 35 °C. 2D-NOESY, TOCSY, and DQF-COSY experiments were performed in phase sensitive mode using the "noesytp", "cosydftp", and "mlevtp" pulse programs and Fourier transformed using standard procedures. TOCSY spectra were acquired using a spin–lock field strength of 10 000 Hz and 60 ms duration. 2D-NOESY experiments have a mixing time of 300 ms. FABMS spectra were obtained using Xe as a gas and 3-nitrobenzyl alcohol (positive mode) or diethanolamine (negative mode) as the matrix, unless otherwise stated. Metastable decompositions in the first FFR were recorded by B/E-linked scan technique in both the spontaneous and activated (CAD) mode, with Ar as the collision gas.

Ginsenoside Rg_1 (1): isolated as described in ref 6; mp 190–3 °C; $[\alpha]^{27}_D$ +225 (*c* = 0.5, THF) (lit.[6] mp 194–6 °C; $[\alpha]^{19.5}_D$ +32, Py); R_f 0.25 (AcOEt–MeOH–H_2O, 10:2:0.5); ^1H NMR (300 MHz) δ 0.87 (3 H, s), 0.91 (6 H, s), 0.99 (3 H, s), 1.26 (3 H, s), 1.28 (3 H, s), 1.58 (3 H, s), 1.65 (3 H, s), 2.93 (1 H, dd, *J* = 9.0, 7.5 Hz), 2.98 (1 H, dd, *J* = 11.5, 5.5 Hz), 3.05 (1 H, dd, *J* = 9.0, 7.8 Hz), 3.10–3.15 (4 H, m), 3.25 (2 H, t, *J* = 9.0 Hz), 3.47 and 3.48 (each 1 H, dd, *J* = 10.0, 5.0 Hz), 3.55 (1 H, td, *J* = 10.0, 5.0 Hz), 3.65 (2 H, dd, *J* = 10.0, 2.0 Hz), 3.95 (1 H, td, *J* = 11.0, 3.5 Hz), 4.27 (1 H, d, *J* = 7.8 Hz), 4.49 (1 H, d, *J* = 7.5 Hz), 5.10 (1 H, bt, *J* = 7.0 Hz); ^{13}C NMR (75.2 MHz) δ 15.52, 16.95 (double), 17.12, 17.50, 21.78, 22.37, 25.31, 25.90, 26.90, 30.16, 30.43, 30.86, 35.61, 38.81, 39.03, 39.99, 40.44, 44.20, 48.45, 49.07, 50.76, 50.80, 60.45, 61.69 (double), 69.24, 70.70 (double), 74.09, 74.28, 76.51 (double), 77.40, 77.60, 77.82, 78.58, 82.34, 96.79, 104.25, 125.40, 130.11; FABMS *m/z* 799 $(M - H)^-$, 637, 619, 475.

Enzymatic Esterification of Ginsenoside Rg_1 (1) with Vinyl Acetate. **1** (100 mg) was dissolved in 3.5 mL of *tert*-amyl alcohol, vinyl acetate (1.5 mL) and *C. a.* B lipase (300 mg) were added, and the suspension was shaken at 45 °C for 6 h. The enzyme was filtered off, the solvent evaporated, and the crude residue purified by flash chromatography (eluent: AcOEt–MeOH–H_2O, 10:2:0.5) to give 5 mg of 6′,6″-di-*O*-acetyl ginsenoside Rg_1 (**1**, 5%) and 92 mg of 6′-*O*-acetyl ginsenoside Rg_1 (**1b**, 87%).

1a: mp 169–172 °C; R_f 0.46 (AcOEt–MeOH–H_2O, 10:2:0.5); ^1H NMR (300 MHz) δ 0.87 (3 H, s), 0.91 (3 H, s), 0.92 (3 H, s), 1.00 (3 H, s), 1.28 (6 H, s), 1.57 (3 H, s), 1.66 (3 H, s), 2.00 (3 H, s), 2.02 (3 H, s), 2.96 (1 H, dd, *J* = 11.5, 5.5 Hz), 2.97 (1 H, dd, *J* = 9.0, 7.5 Hz), 3.07 (1 H, dd, *J* = 9.0, 7.8 Hz), 3.09 (2 H, t, *J* = 9.0 Hz), 3.24 (1 H, t, *J* = 9.0 Hz), 3.26 (1 H, t, *J* = 9.0 Hz), 3.37 (1 H, ddd, *J* = 9.0, 6.5, 2.0 Hz), 3.39 (1 H, ddd, *J* = 9.0, 6.5, 2.0 Hz), 3.56 (1 H, td, *J* = 11.0, 5.5 Hz), 3.95 (1 H, td, *J* = 11.0, 3.5 Hz), 3.96 (1 H, dd, *J* = 11.5, 6.5 Hz), 4.01 (1 H, dd, *J* = 11.5, 2.0 Hz), 4.31 (1 H, dd, *J* = 11.5, 2.0 Hz), 4.34 (1 H, d, *J* = 7.8 Hz), 4.37 (1H, dd, *J* = 11.5, 2.0 Hz), 4.49 (1H, d, *J* = 7.5 Hz), 5.08 (1 H, bt, *J* = 7.0 Hz); ^{13}C NMR (75.2 MHz) δ 14.10, 15.63, 16.87 (double), 17.48, 20.63 (double), 21.28, 22.10, 25.45, 25.60, 26.91, 29.99 (double), 30.58, 35.35, 38.80, 39.07, 39.90, 40.15, 44.22, 48.06, 48.77, 50.50, 50.60, 60.10, 63.80, 64.10, 68.95, 69.90, 70.10, 73.30, 73.59, 73.70, 76.70, 76.90, 77.10, 78.40, 82.35, 96.32, 104.11, 125.17, 130.30, 170.30 (double); FABMS *m/z* 907 $(M + Na)^+$, 885 $(M + H)^+$, 865, 847, 843, 825, 663, 645, 441, 423; FABMS *m/z* 883 $(M - H)^-$, 841, 823, 799, 781, 679, 661, 637, 619, 475.

1b: mp 179–181 °C; $[\alpha]^{27}_D$ +262 (*c* = 0.5, THF); R_f 0.36 (AcOEt–MeOH–H_2O, 10:2:0.5); ^1H (600 MHz) and ^{13}C NMR (150 MHz) see Table 1; FABMS *m/z* 881 $(M + K)^+$, 865 $(M + Na)^+$, 843 $(M + H)^+$, 685, 663, 645, 441, 423, 409; FABMS *m/z* 841 $(M - H)^-$, 799, 781, 679, 637, 619, 475.

Ginsenoside F_1 (2). **1** (300 mg) was dissolved in 50 mL of 50 mM phosphate buffer at pH 3.8. Hesperidinase (8 g, about 80 units) was added, and the solution was left at 30 °C for 3 days. After liophylization, the residue was purified by flash chromatography to give 49 mg (22%) of ginsenoside F_1 (**2**): mp 142–5 °C; $[\alpha]^{27}_D$ +344 (*c* = 0.1, MeOH–H_2O, 1:1) (lit.[10a] $[\alpha]_D$ +366 (*c* = 1.12, MeOH)); R_f 0.42 (AcOEt–MeOH–H_2O, 10:2:0.5); ^1H NMR (300 MHz) δ 0.87 (3 H, s), 0.89 (6 H, s), 1.00 (3 H, s), 1.25 (3 H, s), 1.29 (3 H, s), 1.58 (3 H, s), 1.67 (3 H, s), 2.94 (1 H, dd, *J* = 8.5, 7.9 Hz), 2.98 (1 H, dd, *J* = 11.0, 5.5 Hz), 3.10–3.20 (3 H, m), 3.48 (1 H, dd, *J* = 12.0, 7.0 Hz), 3.56 (1 H, td, *J* = 11.0, 5.0 Hz), 3.66 (1 H, dd, *J* = 12.0, 2.0 Hz), 3.91 (1 H, td, *J* = 11.0, 3.5 Hz), 4.48 (1 H, d, *J* = 7.9 Hz), 5.10 (1 H, bt, *J* = 7.0 Hz); ^{13}C NMR (75.2 MHz) δ 15.76, 17.17 (triple), 17.40, 21.70, 22.32, 25.40, 25.85, 27.04, 30.22 (double), 31.17, 35.45, 38.63, 39.20, 40.05, 40.15, 46.41, 48.25, 48.87, 50.64 (double), 60.50, 61.34, 66.42, 69.05, 70.34, 73.90, 76.52, 77.20 (double), 82.21, 96.67, 125.35, 130.50; FABMS *m/z* 637 $(M - H)^-$, 475.

6′-*O*-Acetyl Ginsenoside F_1 (2a). **2** (30 mg) was dissolved in 1.5 mL of *tert*-amyl alcohol containing 0.5 mL of vinyl acetate. *C. a.* B lipase (100 mg) was added, and the suspension was shaken at 45 °C for 48 h. Usual workup and purification by flash chromatography (eluent: AcOEt–MeOH–H_2O, 10:0.5:0.25) gave 9 mg (28%) of 6′-*O*-acetyl ginsenoside F_1 (**2a**): mp 133–35 °C; R_f 0.56 (AcOEt–MeOH–H_2O, 10:2:0.5); ^1H NMR (300 MHz) δ 0.87 (3 H, s), 0.89 (6 H, s), 1.00 (3 H, s), 1.26 (3 H, s), 1.27 (3 H, s), 1.59 (3 H, s), 1.67 (3 H, s), 2.95–3.05 (2 H, m), 3.12 (1 H, dd, *J* = 9.0, 8.5 Hz), 3.26 (1 H, t, *J* = 8.5 Hz), 3.37 (1 H, ddd, *J* = 9.0, 7.0, 2.0 Hz), 3.59 (1 H, td, *J* = 11.0, 5.0 Hz), 3.91 (1 H, td, *J* = 11.0, 3.5 Hz), 4.02 (1 H, dd, *J* = 11.5, 7.0 Hz), 4.32 (1 H, dd, *J* = 11.5, 2.0 Hz), 4.49 (1 H, d, *J* = 7.9 Hz), 5.10 (1 H, bt, *J* = 7.0 Hz); ^{13}C NMR (75.2 MHz) δ 15.81, 16.92 (double), 17.12, 17.50, 20.60, 21.29, 22.10, 25.51, 25.75, 27.05, 30.08 (double), 31.10, 35.33, 38.44, 38.69, 40.07, 40.23, 46.29, 48.05, 48.72, 50.57, 50.70, 60.35, 63.84, 66.40,

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69.03, 70.30, 73.30, 73.75, 76.99 (double), 82.25, 96.40, 125.27, 130.20, 170.18; FABMS *m/z* 679 (M − H)$^-$, 637, 475.

Enzymatic Esterification of Ginsenoside Rg$_1$ (1) with Methyl 2,2,2-Trifluoroethyl Malonate. **1** (200 mg) was dissolved in 10 mL of *tert*-amyl alcohol containing 1 mL of methyl 2,2,2-trifluoroethyl malonate.[16] *C. a.* B lipase (1 g) was added and the suspension shaken at 45 °C for 24 h. Usual workup and purification by flash chromatography (eluent: AcOEt−MeOH−H_2O, 10:2:0.5) gave 52 mg (21%) of 6′-(*O*)-((2,2,2-trifluoroethoxy)carbonyl)acetyl ginsenoside Rg$_1$ (**1c**) and 108 mg (48%) of 6′-*O*-(methoxycarbonyl)acetyl ginsenoside Rg$_1$ (**1d**).

1c: mp 136−9 °C; R_f 0.29 (AcOEt−MeOH−H_2O, 8:0.5:0.2); ^{1}H NMR (300 MHz) δ 0.87 (3 H, s), 0.90 (3 H, s), 0.91 (3 H, s), 1.00 (3 H, s), 1.27 (3 H, s), 1.28 (3 H, s), 1.59 (3 H, s), 1.66 (3 H, s), 2.94 (1 H, dd, J = 9.0, 8.0 Hz), 2.98 (1 H, dd, J = 11.5, 5.5 Hz), 3.06 (1 H, dd, J = 9.0, 7.8 Hz), 3.10−3.15 (3 H, m), 3.24 (2 H, t, J = 9.0 Hz), 3.39 (1 H, ddd, J = 9.0, 6.5, 2.0 Hz), 3.47 (1 H, dd, J = 11.0, 6.0 Hz), 3.57 (1 H, td, J = 11.0, 5.5 Hz), 3.66 (1 H, dd, J = 11.0, 2.0 Hz), 3.68 (2 H, s), 3.95 (1 H, td, J = 11.0, 3.5 Hz), 4.07 (1 H, dd, J = 11.5, 6.5 Hz), 4.35 (1 H, d, J = 7.8 Hz), 4.47 (1 H, dd, J = 11.5, 2.0 Hz), 4.49 (1 H, d, J = 7.5 Hz), 4.73 (2 H, q, J = 8.5 Hz), 5.10 (1 H, bt, J = 7.0 Hz); FABMS (gly + thiogly) *m/z* 1075 (967 + thiogly)$^-$, 975 (867 + thiogly)$^-$, 967 (M − H)$^-$, 867.

1d: mp 153−4 °C; $[\alpha]^{27}_D$ +166 (c = 0.5, THF); R_f 0.22 (AcOEt−MeOH−H_2O, 8:0.5:0.2); ^{1}H NMR (300 MHz) δ 0.87 (3 H, s), 0.90 (3 H, s), 0.91 (3 H, s), 1.00 (3 H, s), 1.26 (3 H, s), 1.27 (3 H, s), 1.59 (3 H, s), 1.66 (3 H, s), 2.94 (1 H, dd, J = 9.0, 8.0 Hz), 2.98 (1 H, dd, J = 11.5, 5.5 Hz), 3.06 (1 H, dd, J = 9.0, 7.8 Hz), 3.07−3.18 (5 H, m), 3.24 (2 H, t, J = 9.0 Hz), 3.38 (1 H, ddd, J = 9.0, 6.5, 2.0 Hz), 3.47 (1 H, dd, J = 11.0, 6.0 Hz), 3.56 (1 H, td, J = 11.0, 5.5 Hz), 3.65 (1 H, dd, J = 11.0, 2.0 Hz), 3.94 (1 H, td, J = 11.0, 3.5 Hz), 4.07 (1 H, dd, J = 11.5, 6.5 Hz), 4.34 (1 H, d, J = 7.8 Hz), 4.46 (1 H, dd, J = 11.5, 2.0 Hz), 4.48 (1 H, d, J = 7.5 Hz), 5.10 (1 H, bt, J = 7.0 Hz); ^{13}C NMR (75.2 MHz) δ 15.58, 16.85 (triple), 17.47, 21.48, 22.11, 25.40, 25.63, 26.73, 29.99 (double), 30.55, 35.18, 38.76, 38.96, 39.79, 40.08, 40.72, 44.25, 48.07, 48.75, 50.37, 50.44, 52.20, 60.17, 61.15, 64.83, 69.78, 69.89, 70.11, 73.46, 73.81 (double), 76.53, 77.16 (double), 77.18, 78.18, 81.96, 96.51, 104.10, 125.23, 130.11, 166.80, 167.0; FABMS *m/z* 899 (M − H)$^-$, 867, 799, 637, 619, 475.

Enzymatic Esterification of Ginsenoside Rg$_1$ (1) with Dimethyl Malonate. **1** (200 mg) was dissolved in 15 mL of a 1:1 mixture of *tert*-amyl alcohol and dimethyl malonate. *C. a.* B lipase (1 g) was added and the suspension shaken at 45 °C for 24 h. Usual workup and purification by flash chromatography (AcOEt−MeOH−H_2O, 10:2:0.5) gave 193 mg (86%) of 6′-*O*-(methoxycarbonyl)acetyl ginsenoside Rg$_1$ (**1d**).

Enzymatic Esterification of Ginsenoside Rg$_1$ (1) with Bis(2,2,2-trichloroethyl) Malonate. **1** (200 mg) was dissolved in 7 mL of *tert*-amyl alcohol. Bis(2,2,2-trichloroethyl) malonate[16] (1.15 g, 10 equiv) and *C. a.* B lipase (1 g) were added, and the suspension was shaken at 45 °C for 43 h. The solvent was evaporated, the oily residue washed with diethyl ether, and the crude solid residue purified by flash chromatography (eluent: AcOEt−MeOH−H_2O, 8.5:1.5:0.2) to give 181 mg (71%) of 6′-*O*-((2,2,2-trichloroethoxy)carbonyl)acetyl ginsenoside Rg$_1$ (**1f**): mp 148−150 °C; $[\alpha]^{27}_D$ +150 (c = 0.1, THF); R_f 0.52 (AcOEt−MeOH−H_2O, 8:2:0.5); ^{1}H NMR (300 MHz) δ 0.87 (3 H, s), 0.90 (3 H, s), 0.91 (3 H s), 1.01 (3 H, s), 1.26 (3 H, s), 1.28 (3 H, s), 1.60 (3 H, s), 1.66 (3 H, s), 2.94 (1 H, dd, J = 9.0, 7.5 Hz), 2.97 (1 H, dd, J = 11.5, 5.5 Hz), 3.06 (1 H, dd, J = 9.0, 7.8 Hz), 3.10−3.15 (3 H, m), 3.24 (2 H, t, J = 9.0 Hz), 3.39 (1 H, ddd, J = 9.0, 6.5, 2.0 Hz), 3.47 (1 H, dd, J = 11.5, 6.5 Hz), 3.57 (1 H, td, J = 11.0, 5.5 Hz), 3.64, 3.70 (each 1 H, AB system, J = 20 Hz), 3.66 (1 H, dd, J = 11.5, 2.0 Hz), 3.94 (1 H, td, J = 11.0, 3.5 Hz), 4.08 (1 H, dd, J = 11.5, 6.5 Hz), 4.34 (1 H, d, J = 7.8 Hz), 4.47 (1 H, dd, J = 11.5, 2.0 Hz), 4.49 (1 H, d, J = 7.5 Hz), 4.90 (2 H, s), 5.09 (1 H, bt, J = 7.0 Hz); ^{13}C NMR (75.2 MHz) δ 15.45, 16.76 (triple), 17.38, 21.52, 22.14, 25.24, 25.60, 26.58, 29.96 (double), 30.55, 35.15, 38.75, 38.97, 39.90, 40.10 (double), 44.16, 48.07, 48.75, 50.54, 50.64, 60.18, 61.12, 64.98, 68.86, 69.67, 70.10, 73.45, 73.73 (triple), 76.32, 76.94 (double), 77.00, 78.20, 82.10, 96.47, 103.96, 125.15, 130.03, 165.10, 166.20 (CCl_3 not evident); FABMS *m/z* (matrix, 3-nitrobenzyl alcohol + diethanolamine + methanol) 1125 (1020 + diethanolamine or 972 + 3-nitrobenzyl alcohol)$^-$, 1020 (Rg$_1$−OCOCH$_2$COO−CH$_2$C$_6$H$_4$−NO$_2$ − H)$^-$, 1015 (M − H, ^{35}Cl)$^-$, 972 (Rg$_1$−OCOCH$_2$COON(CH$_2$CH$_2$OH)$_2$ − H)$^-$, 899 (Rg$_1$OCOCH$_2$COOCH$_3$ − H)$^-$, 841, 799.

6′-*O*-Carboxyacetyl Ginsenoside Rg$_1$ (1e). (a) **1d** (50 mg) was dissolved in 1 mL of DMF and the solution added to 9 mL of 0.1 M phosphate buffer pH 7 containing 50 μL of pig liver esterase suspension. The solution was left at room temperature for 24 h, water evaporated under reduced pressure (without heating), and the residue purified by flash chromatography (AcOEt−MeOH−H_2O, 10:4:1) to give 12 mg (25%) of 6′-*O*-carboxyacetyl ginsenoside Rg$_1$ (**1e**).

(b) **1f** (100 mg) was dissolved in 10 mL of AcOH, the solution was cooled to about 10 °C, and 100 mg of zinc dust was added. The suspension was stirred until the starting substrate totally disappeared (about 24 h). Zinc dust was filtered off, the solvent evaporated under vacuum without heating, and the residue purified by flash chromatography (AcOEt−MeOH−H_2O, 10:4:1) to give 51 mg (58%) of 6′-*O*-carboxyacetyl ginsenoside Rg$_1$ (**1e**).

1e: mp 193−5 °C; $[\alpha]^{27}_D$ +107 (c = 0.28, H_2O−Py, 14:1); R_f 0.25 (AcOEt−MeOH−H_2O, 10:4:1); ^{1}H NMR (300 MHz, 22 °C) δ 0.88 (3 H, s), 0.90 (6 H, s), 0.98 (3 H, s), 1.26 (3 H, s), 1.27 (3 H, s), 1.59 (3 H, s), 1.66 (3 H, s), 2.89 (1 H, dd, J = 9.0, 8.0 Hz), 2.95−3.12 (7 H, m), 3.19 (2 H, t, J = 9.0 Hz), 3.63 (1 H, dd, J = 11.0, 2.0 Hz), 3.91 (1 H, td, J = 11.0, 3.5 Hz), 4.03 (1 H, dd, J = 11.5, 6.5 Hz), 4.29 (1 H, d, J = 7.8 Hz), 4.43 (2 H, broad d), 5.09 (1 H, bt, J = 7.0 Hz); ^{13}C NMR (75.2 MHz, 22 °C) δ 15.62, 16.95 (triple), 17.62, 21.59, 22.35, 25.53, 25.70, 26.83, 30.07 (double), 30.63, 35.35, 38.95 (double), 39.78, 40.06 (triple), 44.24, 48.14, 48.34, 50.41, 50.62, 60.18, 61.14, 64.35, 69.98, 70.07 (double), 73.59, 73.87 (double), 76.45, 77.20 (triple), 78.36, 82.04, 96.58, 104.19, 125.31, 130.20, 169.90, 170.27; FABMS *m/z* 885 (M − H)$^-$, 841, 799, 679, 619. ^{1}H and ^{13}C NMR spectra at 80 °C were identical with those of 6′-*O*-acetyl ginsenoside Rg$_1$.

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of compounds (20 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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