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Full Paper

Synthesis of Carbohydrate-based Natural Products from *Leonurus japonicus* and their Biological Evaluation as Anti-oxidants

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Natural products are important materials that have found a wide variety of uses, especially in medicine. Traditional Chinese medicine (TCM) has especially taken advantage of natural products and compounds found in *Leonurus*, a species of herb used extensively in TCM to treat various ailments. Herein we describe the synthesis of three natural products from *Leonurus japonicus* and our investigation of their hepatoprotective properties.

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Introduction

Natural products are important both historically and currently as people rely on nature as a means to every end, from food and shelter to clothing and tools. Furthermore, nature was once the only source of treatments for a wide variety of diseases through the use of traditional medical systems.[1] Although there are now many techniques in modern medicine, nature continues to represent a valuable resource of materials for medical research. Traditional medical systems use methods of preparing and testing therapeutics, which differ greatly from modern techniques. Even though traditional therapeutics may not be immediately translatable to modern medicines, they still may represent very useful starting points for drug discovery.[2] Traditional Chinese Medicine (TCM) dates back to 1100 BCE and is an example of a traditional medical system based on the systematic testing of natural materials, which has been well documented for thousands of years.[3] As such, TCM has been used as a starting point for investigating natural products that may be useful for drug development.

Leonurus is a specific group of herbs that are highly important in TCM.[4] This genus contains numerous species that can be found throughout Europe and Asia, as well as in some regions of America and Africa, where these species have been incorporated in traditional medicine systems.[4] The history and copious use of *Leonurus* herbs in traditional medicine have made them interesting candidates for modern scientific research. Numerous species of *Leonurus* have been used to study the effects of the intrinsic natural products, and extracts of various species have shown that *Leonurus* contains biologically active labdane-type diterpenoids, iridoids, flavonoids and flavonoid glycosides, cyclic peptides, alkaloids, and phenylethyl glycosides.[5,6] Of interest to us was a recent study by Li et al., who examined the carbohydrate component of *Leonurus japonicus* and reported that four compounds Cistanoside E **1**, Leonoside F **2**, Leonoside E **3**, and Verbascoside **4** (Fig. 1) exhibited hepatoprotective properties.[6] Cistanoside E **1** and Verbascoside **4** have been previously isolated [7,8] from other plant materials; the synthesis of Verbascoside **4** has been reported.[7] However, despite the biological activity of Cistanoside E **1**, its chemical synthesis has yet to be reported. In the present study, Cistanoside E **1**, Leonoside F **2**, and Leonoside E **3** were selected as candidates for synthesis to study their biological properties further. Recently, the synthesis of Leonoside F **2** and Leonoside E **3** has been reported,[9] however, no rigorous studies relating to the biological activity of these compounds were discussed. Ggiven the importance of access to these compounds for biological studies, it seemed to us that a route for the preparation of them was warranted.

Results and Discussion

Our attention was first directed to the synthesis of **1** and **2**, and we identified the disaccharide **5** was as the key intermediate in the synthesis of both molecules. The disaccharide **5**[10] was easily prepared from the allyl glycoside **6**[11,12] and trichloroacetimidate **7**[13] using the procedures reported in the literature (Scheme 1).

The disaccharide **5** was then treated with glacial acetic acid to yield the diol **8** in good yield. For the second glycosylation (towards the synthesis of Leonoside F **2**), the diol was treated with *tert*-butyldimethylsilyl chloride, producing the silyl ether **9** in excellent yield. Acetylation of **9** using acetic anhydride in pyridine gave the fully protected disaccharide **10**. Finally, removal of the silyl ether with tetrabutylammonium fluoride from **10** gave the alcohol **11** in excellent yield. For the synthesis

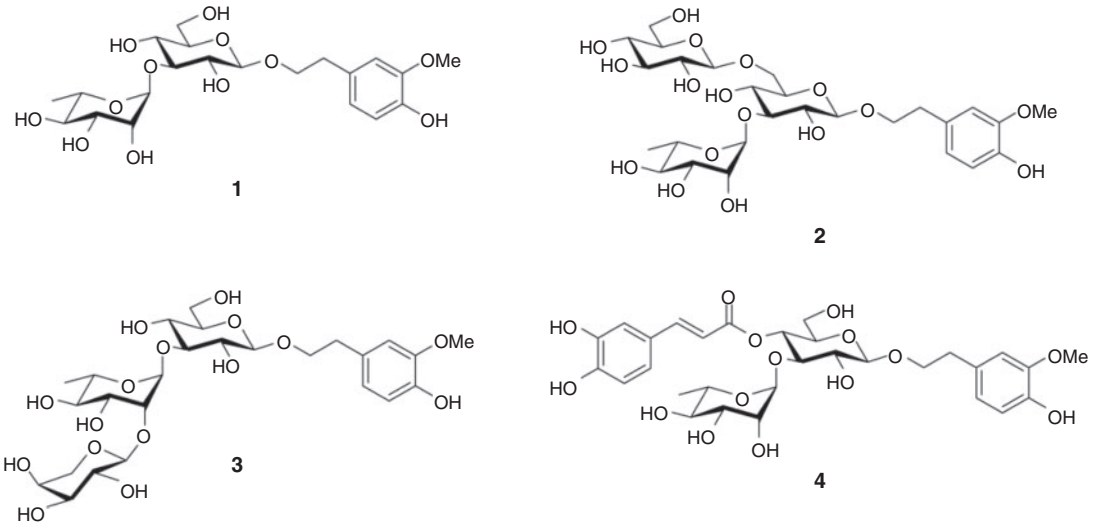
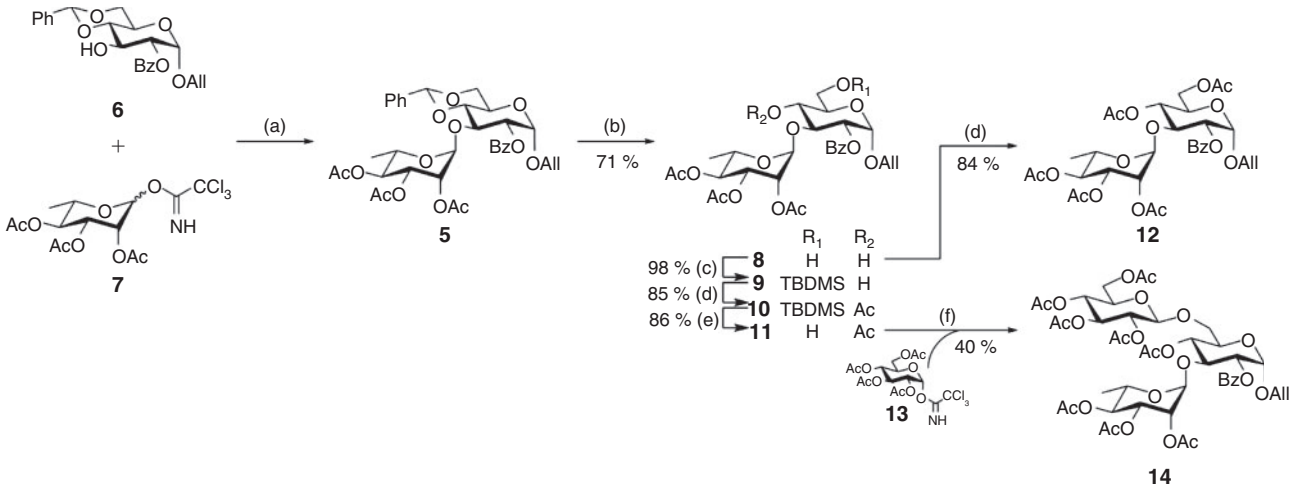


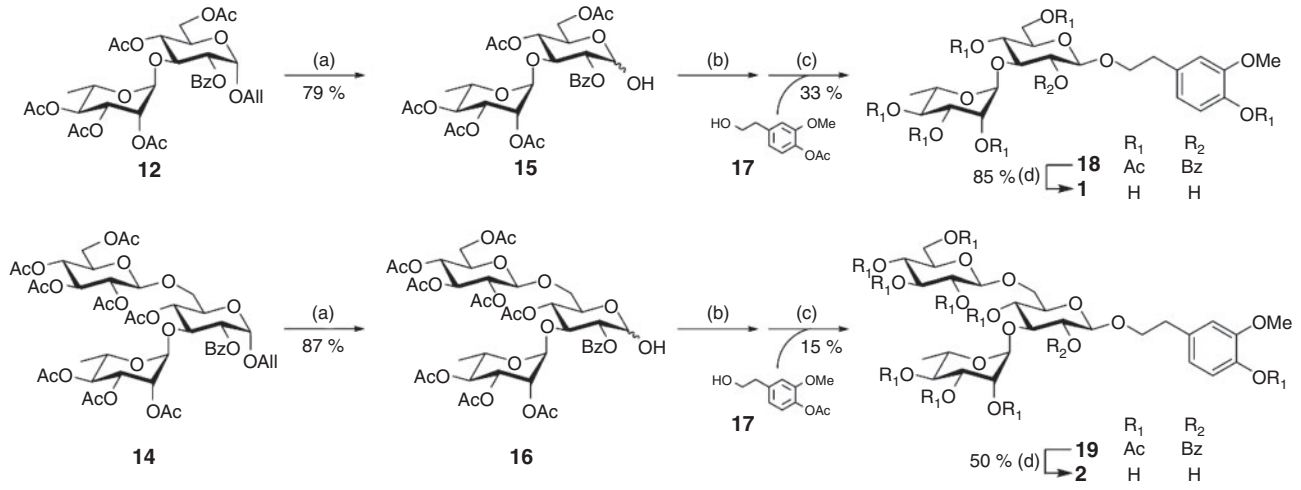
Fig. 1. Carbohydrate-based hepatoprotective compounds from *Leonurus japonicus.*



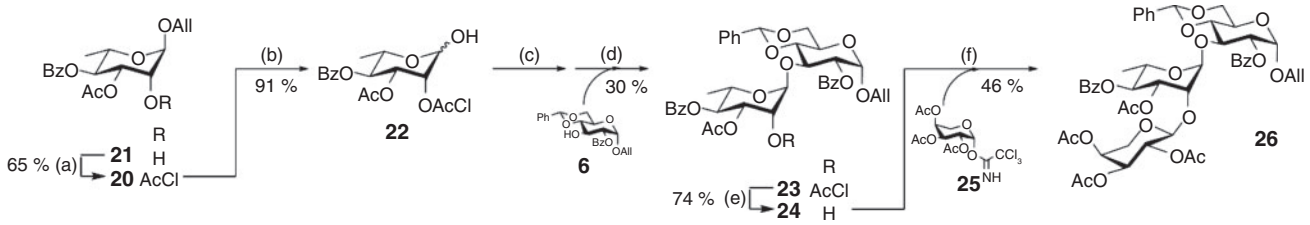
Scheme 1. (a) TMSOTf, CH_2Cl_2. (b) CH_3COOH/H_2O (4 : 1). (c) TBDMSCl, DMAP, CH_2Cl_2. (d) Ac_2O, C_5H_5N. (e) TBAF, CH_3COOH, THF. (f) TMSOTf, CH_2Cl_2.

of Cistanoside E **1**, the diol **8** was acetylated directly to give the benzoate **12**. The alcohol **11** was then treated with the trichloroacetimidate **13**[14] under the conditions employed for glycosylation to give the trisaccharide **14** in good yield. With both the desired compounds **12** and **14** in hand, attention was directed towards the removal of their respective allyl glycosides. A procedure involving palladium(II) chloride in methanol, a standard method for the removal of allyl ethers,[12] was first attempted using **12**. However, this method resulted in the formation of several products, as identified by thin layer chromatography (TLC). A significant decrease in pH was also observed. Based on this observation, it was rationalised that both deacetylation and allyl glycoside removal were occurring. Because it was necessary to perform the reaction on compounds in the presence of acetyl groups, a different method was investigated. It was hoped that the conditions of Ban and Mrksich,[15] in which a solution of sodium acetate and acetic acid is used as a buffer, would aid in the formation of the desired hemiacetal. Gratifyingly, the hemiacetal **15** was obtained in good yield in the absence of by-products; hemiacetal **16** was also successfully synthesized from **14** (Scheme 2). Hemiacetals **15** and **16** were then converted to their corresponding trichloroacetimidates. The latter were then used in situ as glycosyl donors for the glycosylation of the protected homovanillyl alcohol derivative **17**[16] to yield **18** and **19**, respectively. Finally, the protecting groups were removed using sodium methoxide in methanol to give Cistanoside E **1** and Leonoside F **2** in good yields (85 % and 50 %, respectively). The ^1H and ^{13}C NMR spectra for the prepared compounds were consistent with those observed from natural sources of **1**[8] and **2**,[6] and synthetic sources for **2**.[9]

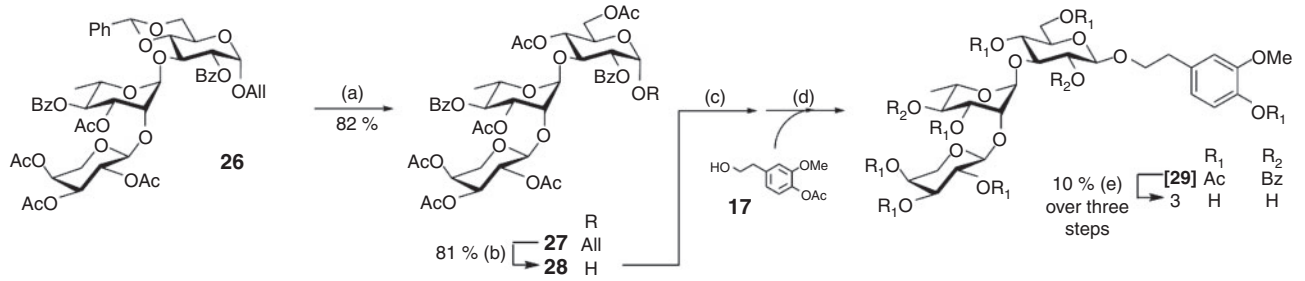
We then turned our attention to the synthesis of Leonoside E **3**, which differs from the synthesis of Cistanoside E **1** and Leonoside F **2**. We felt that a suitably prepared L-rhamnose derivative **20** could be used as a linker molecule to allow for glycosylation to the parent glucose moiety of Leonoside E **3**, and the chloroacetyl moiety at *O*-2 of **20** could be selectively removed, with the resultant alcohol, in turn, used as a glycosyl acceptor for the arabinose portion of the molecule. Thus, using procedures described in the literature,[17] the alcohol **21** was prepared from L-rhamnose in good yield (Scheme 3). Treatment of **21** with chloroacetyl chloride under basic conditions gave the desired allyl glycoside **20** in good yield. Using the conditions described above, the hemiacetal **22** was prepared from **20** in



Scheme 2. (a) $PdCl_2$, CH_3COOH, NaOAc, H_2O. (b) CCl_3CN, DBU, CH_2Cl_2. (c) TMSOTf, CH_2Cl_2. (d) NaOMe, MeOH.



Scheme 3. (a) $ClCH_2COCl$, CH_2Cl_2, C_5H_5N. (b) $PdCl_2$, CH_3COOH, NaOAc, H_2O. (c) CCl_3CN, DBU, CH_2Cl_2. (d) TMSOTf, CH_2Cl_2. (e) $SC(NH_2)_2$, 2,6-lutidine, MeOH, CH_2Cl_2. (f) TMSOTf, CH_2Cl_2.



Scheme 4. (a) i. CH_3COOH/H_2O (4 : 1); ii. Ac_2O, C_5H_5N. (b) $PdCl_2$, CH_3COOH, NaOAc, H_2O. (c) CCl_3CN, DBU, CH_2Cl_2. (d) TMSOTf, CH_2Cl_2. (e) NaOMe, MeOH.

excellent yield. The hemiacetal **22** was then converted to its corresponding trichloroacetimidate and used in situ as a glycosyl donor for the glycosylation of **6** to give the disaccharide **23**. Selective removal of the chloroacetyl group using a procedure involving thiourea and 2,6-lutidine[18] gave the alcohol **24**, which was then treated with the trichloroacetimidate **25**[19] under conditions of glycosylation to give the trisaccharide **26** in good yield.

The benzylidene acetal was subsequently removed from **26**, followed by in situ acetylation to give the diacetate **27**. Using the conditions described above, the hemiacetal **28** was prepared from **27** in excellent yield (Scheme 4). The hemiacetal **28** was then converted to its corresponding trichloroacetimidate and used in situ as a glycosyl donor for the glycosylation of the protected homovanillyl alcohol derivative **17**[16] to give the trisaccharide **29**. The latter compound was slightly contaminated with residual **17** (based on the R_F value) following purification. Finally, the protecting groups were removed using sodium methoxide in methanol, generating Leonoside E **3** in good yield. The ^1H and ^{13}C NMR spectra for the compound were consistent with those observed for the naturally occurring[6] and synthetic[9] compounds.

With the desired compounds in hand, we then directed our attention to studying in more detail the hepatoprotective nature of these compounds. Hepatoprotection is the ability (of a material) to prevent damage to the liver. One pathway that leads to damage within cells, particularly in the liver, is mediated by oxidative stress.[20] Oxygen-free radicals and other reactive oxygen species (ROS) are released as by-products of numerous physiological processes.[21,22] However, excess ROS can result in oxidative stress, as evidenced by DNA degradation, lipid peroxidation, and protein damage.[23] Under conditions of disease, the liver is particularly vulnerable to oxidative stress and recent investigations have shown that oxidant-induced liver injuries are mediated by the direct effects of reactive oxygen species on signal transduction pathways.[24]

Therefore, we assessed the effects of the three compounds **1**–**3**, at various concentrations, on indicators of oxidative stress

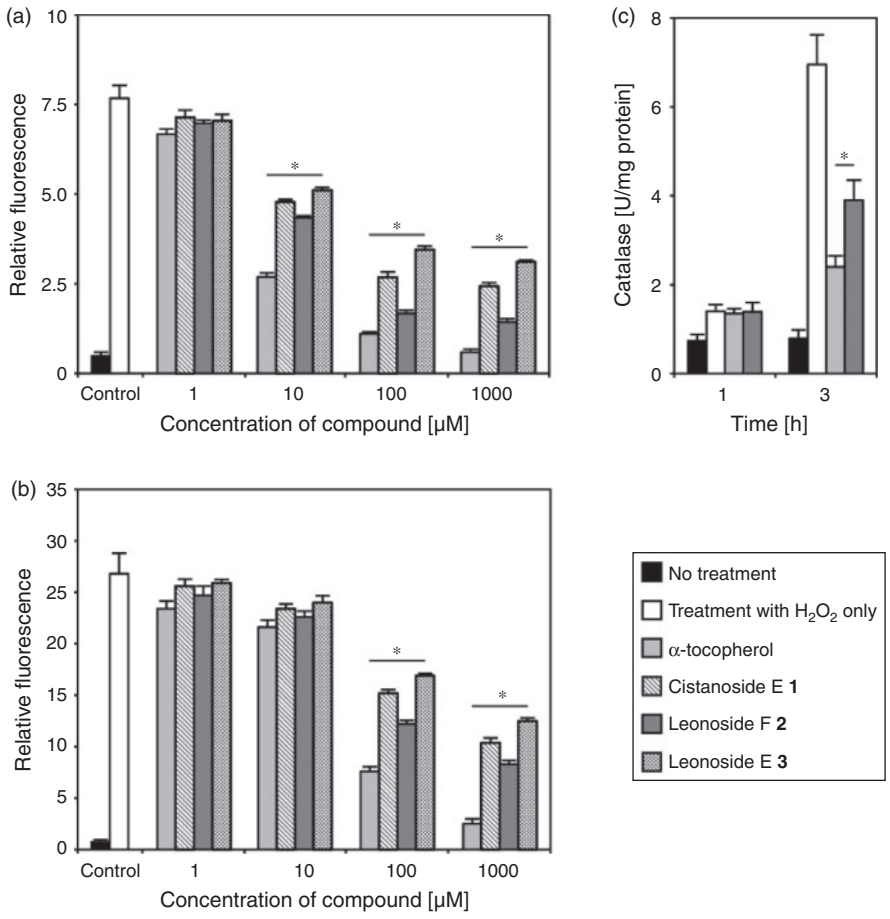


Fig. 2. Effect of compounds **1**–**3** at various concentrations on intracellular ROS production in HepG2 cells in the presence of H_2O_2 at (a) 60 min and (b) 3 h. (c) Effect of compound **2** (100 μM) on catalase activity in the presence of H_2O_2. Results are shown as (mean ± standard deviation) of three replicates; significance of the results relating to treatment with H_2O_2 was determined by ANOVA and Bonferroni–Dunn post hoc tests ($p \leq 0.05$).

in HepG2 cells (Fig. 2a, b). The oxidative stress agent H_2O_2, which has been demonstrated previously as a good model for oxidative stress in liver cells, was used.[25] Upon treatment with H_2O_2, ROS production increased compared with cells that were subjected to no stress at both time points studied (Fig. 2a, b). Cells that had been pretreated with compound **1**–**3** or α-tocopherol, a known inhibitor of ROS generation,[26] were also stressed with H_2O_2 (250 μM), and the amount of ROS was measured. Interestingly, there was a dose-dependent effect for each compound **1**–**3** studied; compound **2** gave the best result not only at both time points, but also at the lowest effective concentration (100 μM). Importantly, there was no loss in cell viability for the compounds tested at the highest concentration (1 mM).

To determine the effect of compound **2** on the activity of endogenous anti-oxidants, the activity of catalase was measured. Catalase is an endogenous anti-oxidant enzyme that plays a pivotal role in preventing cellular damage caused by ROS.[27] We assessed the effects of compound **2** (100 μM) on catalase activity in cells stressed with H_2O_2 over a 3-h period. Relative to untreated controls, enzyme activity decreased in cells exposed to compound **2** (Fig. 2c). These observations are supported by the decrease in ROS formation observed in earlier experiments. It is likely that over the 3-h time period, the amount of enzyme produced in response to H_2O_2 stress is reduced because of a reduction in the concentrations of ROS present, with Leonoside F **2** found to have the best effect in controlling ROS generation.

Conclusion

In conclusion, we have demonstrated new syntheses for three important carbohydrate-based natural products found in *Leonurus* herbs, with the ease of preparation making this route particularly attractive. Using oxidative stress assays we have putatively found reasons for the hepatoprotective properties of these compounds. All compounds were able to reduce oxidative stress, and these results set the stage for further investigations into the biological roles of these molecules, something our laboratory is actively pursuing.

Experimental

^1H and ^{13}C NMR spectra were obtained on a Bruker ARX500 (500 MHz for ^1H and 125.7 MHz for ^{13}C) or a Bruker AV600 (600 MHz for ^1H and 150.8 MHz for ^{13}C) spectrometer. Unless stated otherwise, deuterated chloroform ($CDCl_3$) was used as the solvent with $CHCl_3$ (δ_H 7.26) or $CDCl_3$ (δ_C 77.16) employed as internal standards. For deuteriomethanol (CD_3OD), CD_2HOD (^1H, δ 3.30), or CD_3OD (^{13}C, δ 49.0) were employed as internal standards. Mass spectra were recorded on a Waters LCT Premier XE spectrometer, in W-mode, using the ESI method, with CH_3CN/H_2O (9 : 1) as a matrix. IR spectra were obtained using a PerkinElmer Spectrum One FT-IR spectrometer fitted with a PerkinElmer Universal ATR sampling accessory. Elemental analyses were performed at the Robertson

Microanalytical Facility. Flash chromatography was performed on silica gel (BDH) with the specified solvents. Thin layer chromatography (TLC) was conducted on silica gel 60 F_{254} (Merck) and aluminium-backed plates that were stained by heating (>200°C) with 5 % sulfuric acid in ethanol. Percentage yields for chemical reactions as described are quoted only for compounds that were purified by recrystallization or by column chromatography, and purity was assessed by TLC or ^{1}H NMR spectroscopy.

*Allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl-(1→3)-2-*O*-benzoyl-α-D-glucopyranoside* ***8***

Allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucoside **5**[10] (0.50 g, 0.73 mmol) was treated with CH_3COOH/H_2O (4 : 1, 20 mL) and the resulting solution was stirred at 70°C for 2 h. After the reaction was complete (as assessed by TLC), the reaction mixture was concentrated and the residue co-evaporated with toluene (3 × 25 mL). Flash chromatography of the resulting oil (13 : 7 ethyl acetate (EtOAc) : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.55), **8** as a white foam (0.31 g, 71 %). ν_{max} (neat)/cm^{-1} 3475 (w), 2935 (w), 1745 (s), 1724 (s). δ_H (600 MHz, $CDCl_3$) 8.07–8.04 (m, 2H, Ar), 7.59–7.55 (m, 1H, Ar), 7.46–7.43 (m, 2H, Ar), 5.84–5.77 (m, 1H, C*H*=CH_2), 5.27–5.21, 5.05–4.99 (2 m, 7H, CH=CH_2, H1, H1′, H2, H2′, H3′, H4′), 5.14–5.11 (m, 1H, CH=CH_2), 4.19–4.12, 3.92–3.86, 3.80–3.70 (3 m, 7H, H3, H4, H5, H5′, H6, H6, CH_2), 3.99 (dddd, *J* 1.5, 1.5, 6.0, 13, 1H, CH_2), 3.56 (d, *J* 3, 1H, OH), 2.09 (dd, *J* 5.5, 5.5, 1H, OH), 2.02 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3), 1.89 (s, 3H, C(O)CH_3), 1.25 (d, *J* 6.5, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.0 (C=O), 169.7 (C=O), 166.0 (C=O), 133.5 (*C*H=CH_2), 133.4 (Ar), 130.0 (Ar), 129.5 (Ar), 128.6 (Ar), 117.9 (CH=*C*H_2), 99.3, 95.4 (C1, C1′), 81.9, 72.7, 71.3, 71.1, 70.1, 69.9, 68.5, 67.8 (C2, C2′, C3, C3′, C4, C4′, C5, C5′), 68.8 (CH_2), 62.4 (C6), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 17.6 (CH_3). *m/z* (HR-MS) (ESI) 597.2186; $[M+H]^+$ requires 597.2183.

*Allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl-(1→3)-2-*O*-benzoyl-6-*O*-tert-butyldimethylsilyl-α-D-glucopyranoside* ***9***

tert-Butyldimethylsilyl chloride (0.22 g, 1.5 mmol), 4-dimethylaminopyridine (DMAP; 15 mg) and triethylamine (0.70 mL, 5.0 mmol) were added to a solution of **8** (0.49 g, 0.82 mmol) in CH_2Cl_2 (5 mL) at 0°C, and then stirred at room temperature for 14 h. After the reaction was complete (as assessed by TLC), the reaction mixture was diluted in CH_2Cl_2 (30 mL) and washed with water (20 mL), saturated $NaHCO_3$ solution (3 × 20 mL), and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (1 : 1 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.5, 2 : 3 EtOAc : hexane eluent), **9** as a white foam (0.57 g, 98 %). ν_{max} (neat)/cm^{-1} 2934 (w), 1748 (s). δ_H (500 MHz, $CDCl_3$) 8.05–8.03 (m, 2H, Ar), 7.57–7.54 (m, 1H, Ar), 7.45–7.41 (m, 2H, Ar), 5.84–5.76 (m, 1H, C*H*=CH_2), 5.27–5.20, 5.05–4.99 (2 m, 6H, H1′, H2, H2′, H3′, H4′, CH=CH_2), 5.18 (d, *J* 3.5, 1H, H1), 5.12–5.10 (m, 1H, CH=CH_2), 4.23–4.14, 3.98–3.95, 3.73–3.69 (3 m, 8H, H3, H4, H5, H5′, H6, H6, CH_2), 3.48 (d, *J* 2.5, 1H, OH), 2.02 (s, 3H, C(O)CH_3), 1.97 (s, 3H, C(O)CH_3), 1.89 (s, 3H, C(O)CH_3), 1.22 (d, *J* 6.5, 3H, CH_3), 0.93 (s, 9H, $C(CH_3)_3$), 0.12 (s, 6H, $Si(CH_3)_2$). δ_C (125.7 MHz, $CDCl_3$) 170.1 (C=O), 169.7 (C=O), 169.6 (C=O), 165.9 (C=O), 133.7, 133.3 (*C*H=CH_2, Ar), 130.0 (Ar), 129.6 (Ar), 128.5 (Ar), 117.7 (CH=*C*H_2), 99.2, 95.3 (C1, C1′), 81.0, 73.0, 71.2, 71.2, 71.0, 70.0, 68.7, 68.5, 67.4, 63.8 (C2, C2′, C3, C3′, C4, C4′, C5, C5′, C6, CH_2), 26.1 ($SiC(CH_3)_3$), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 18.5 (Si*C*$(CH_3)_3$), 17.4 (CH_3), −5.2 ($Si(CH_3)_2$), −5.3 ($Si(CH_3)_2$). *m/z* (HR-MS) (ESI) 711.3073; $[M+H]^+$ requires 711.3048.

*Allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl-(1→3)-4-*O*-acetyl-2-*O*-benzoyl-6-*O*-tert-butyldimethylsilyl-α-D-glucoside* ***10***

Acetic anhydride (0.33 mL, 3.5 mmol) was added dropwise to a solution of **9** (0.51 g, 0.72 mmol) in pyridine (5 mL) and stirred at 40°C for 24 h. After the reaction was complete (as assessed by TLC), the reaction mixture was quenched with methanol (MeOH, 10 mL) and concentrated. The residue was suspended in EtOAc (30 mL) and washed with hydrochloric acid (1 M, 2 × 30 mL), saturated $NaHCO_3$ solution (3 × 30 mL), and brine (2 × 30 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (2 : 3 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.5), **10** as a colourless oil (0.46 g, 85 %). ν_{max} (neat)/cm^{-1} 2930 (w), 2857 (w), 1748 (s). δ_H (500 MHz, $CDCl_3$) 8.06–8.03 (m, 2H, Ar), 7.57–7.53 (m, 1H, Ar), 7.45–7.41 (m, 2H, Ar), 5.84–5.77 (m, 1H, C*H*=CH_2), 5.28–5.22 (m, 1H, CH=CH_2), 5.20 (d, *J* 3.7, 1H, H1), 5.14–4.93 (m, 7H, H1′, H2, H2′, H3′, H4, H4′, CH=CH_2), 4.35 (dd, *J* 11.3, 11.3, 1H, H3), 4.21–4.14 (m, 1H, CH_2), 4.00–3.94 (m, 1H, CH_2), 3.89–3.82, 3.67–3.65 (2 m, 4H, H5, H5′, H6, H6), 2.10 (s, 3H, C(O)CH_3), 1.97 (s, 3H, C(O)CH_3), 1.92 (s, 3H, C(O)CH_3), 1.77 (s, 3H, C(O)CH_3), 1.15 (d, *J* 7.5, 3H, CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$). δ_C (125.7 MHz, $CDCl_3$) 170.2 (C=O), 169.6 (C=O), 169.5 (C=O), 169.4 (C=O), 165.5 (C=O), 133.5, 133.3 (*C*H=CH_2, Ar), 130.1 (Ar), 129.4 (Ar), 128.5 (Ar), 117.8 (CH=*C*H_2), 99.4, 94.8 (C1, C1′), 77.7, 73.5, 71.1, 70.9, 70.1, 69.9, 68.6, 68.5, 67.4, 62.9 (C2, C2′, C3, C3′, C4, C4′, C5, C5′, C6, CH_2), 26.0 ($SiC(CH_3)_3$), 21.2 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 18.5 (Si*C*$(CH_3)_3$), 17.5 (CH_3), −5.2 ($Si(CH_3)_2$), −5.3($Si(CH_3)_2$). *m/z* (HR-MS) (ESI) 791.2711; $[M+K]^+$ requires 791.2713.

*Allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnosyl-(1→3)-4-*O*-acetyl-2-*O*-benzoyl-α-D-glucoside* ***11***

A solution of tetra-*N*-butylammonium fluoride (TBAF) in THF (1 M, 0.87 mL, 0.87 mmol) and CH_3COOH (0.87 mL) were added dropwise to a solution of **10** (0.58 g, 0.81 mmol) in THF (2 mL) at 0°C, and stirred at room temperature overnight. The reaction mixture was then concentrated and suspended in EtOAc (30 mL) and washed with water/brine (30 mL, 1 : 1), saturated $NaHCO_3$ solution (30 mL), and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (1 : 1 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.27), **11** as a white foam (0.45 g, 86 %). ν_{max} (neat)/cm^{-1} 3524 (w), 2938 (w), 1746 (s). δ_H (600 MHz, $CDCl_3$) 8.04–8.02 (m, 2H, Ar), 7.56–7.54 (m, 1H, Ar), 7.43–7.41 (m, 2H, Ar), 5.82–5.76 (m, 1H, C*H*=CH_2), 5.26–5.23 (m, 2H, H1, CH=CH_2), 5.13–4.99 (m, 6H, H1′, H2, H2′, H3′, H4, CH=CH_2), 4.94 (dd, *J* 9.6, 9.6, 1H, H4′), 4.40 (dd, *J* 9.6, 9.6, 1H, H3), 4.16 (ddd, *J* 1.2, 5.4, 13.2, 1H, CH_2), 3.98 (ddd, *J* 1.8, 6.0, 13.2, 1H, CH_2), 3.91–3.86 (m, 1H, H5′), 3.78 (ddd, *J* 2.4, 4.2, 10.2, 1H, H5), 3.71–3.67 (m, 1H, H6), 3.59 (ddd, *J* 4.8, 4.8, 13.2, 1H, H6), 2.46 (dd, *J* 5.4, 8.4, 1H, OH), 2.13 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3), 1.93 (s, 3H, C(O)CH_3), 1.78 (s, 3H, C(O)CH_3), 1.16 (d, *J* 6.0, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.8 (C=O), 170.1 (C=O), 169.5 (C=O), 169.4 (C=O), 165.5 (C=O), 133.4 (*C*H=CH_2), 133.3 (Ar), 130.0 (Ar),

129.3 (Ar), 128.5 (Ar), 118.0 (CH=*C*H_2), 99.1, 95.3 (C1, C1′), 76.7 (C3), 73.3, 71.0, 70.0, 69.9, 69.8, 68.5 (C2, C2′, C3′, C4, C4′, C5), 68.9 (CH_2), 67.3 (C5′), 61.3 (C6), 21.0 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 17.4 (CH_3). *m/z* (HR-MS) (ESI) 677.1811; $[M+K]^+$ requires 677.1848.

Allyl 2,3,4-tri-O-acetyl-α-L-rhamnosyl-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-α-D-glucoside **12**

Acetic anhydride (0.27 mL, 2.9 mmol) was added to a solution of the diol **8** (0.55 g, 0.92 mmol) in pyridine (2 mL) and CH_2Cl_2 (4 mL) at 0°C, and the resulting solution was stirred at room temperature for 1 h. After the reaction was complete (as assessed by TLC), the reaction mixture was quenched with MeOH (10 mL) and concentrated. The residue was suspended in EtOAc (30 mL) and washed with hydrochloric acid (1 M, 3 × 20 mL), water (20 mL), saturated $NaHCO_3$ solution (3 × 20 mL), and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (EtOAc : hexane = 2 : 3) afforded, after concentration of the appropriate fractions (R_F 0.45), **12** as a colourless oil (0.53 g, 84 %). ν_{max} (neat)/cm^{-1} 2983 (w), 1742 (s). δ_H (500 MHz, $CDCl_3$) 8.06–8.04 (m, 2H, Ar), 7.58–7.54 (m, 1H, Ar), 7.45–7.42 (m, 2H, Ar), 5.84–5.76 (m, 1H, C*H*=CH_2), 5.30–5.09, 5.02–4.91 (2 m, 9H, H1, H1′, H2, H2′, H3′, H4, H4′, CH=CH_2), 4.35 (dd, *J* 9.5, 9.5, 1H, H3), 4.24 (dd, *J* 4.5, 12.5, 1H, H6), 4.18–4.08, 4.01–3.97 (2 m, 4H, H5, H6, CH_2), 3.90–3.82 (m, 1H, H5′), 2.12 (s, 3H, C(O)CH_3), 2.11 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3), 1.94 (s, 3H, C(O)CH_3), 1.78 (s, 3H, CH_3), 1.16 (d, *J* 6.0, 3H). δ_C (125.7 MHz, $CDCl_3$) 171.0 (C=O), 170.1 (C=O), 169.6 (C=O), 169.5 (C=O), 169.4 (C=O), 165.5 (C=O), 133.4, 133.2 (*C*H=CH_2, Ar), 130.1 (Ar), 129.2 (Ar), 128.5 (Ar), 118.2 (CH=*C*H_2), 99.4, 95.3 (C1, C1′), 77.3, 73.2, 71.0, 69.9, 69.2, 69.1, 68.5, 67.9, 67.4, 62.2, (C2, C2′, C3, C3′, C4, C4′, C5, C5′, C6, CH_2), 21.1 (C(O)*C*H_3), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 17.5 (CH_3). *m/z* (HR-MS) (ESI) 681.2389; $[M+H]^+$ requires 681.2395. Anal. Calc. for $C_{32}H_{40}O_{16}$: C 56.47, H 5.92. Found: C 56.22, H 5.98 %.

Allyl 2,3,4-tri-O-acetyl-α-L-rhamnosyl-(1→3)-[2,3,4,6-tetra-O-acetyl-β-D-glucosyl-(1→6)-]-4-O-acetyl-2-O-benzoyl-α-D-glucoside **14**

A mixture of **11** (0.40 g, 0.63 mmol), **13**[14] (0.39 g, 0.80 mmol), and dry 4-Å molecular sieves (0.80 g) in CH_2Cl_2 (12 mL) were stirred under argon for 15 min, then cooled to −30°C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 75 µL) was then added, and after 5 min at −30°C, stirred at room temperature for 2 h. After the reaction was complete (as assessed by TLC), the reaction mixture was cooled to 0°C, quenched with triethylamine (100 µL), filtered through celite, and concentrated. Flash chromatography of the resulting oil (EtOAc : hexane = 1 : 1) afforded, after concentration of the appropriate fractions (R_F 0.44, 3 : 2 EtOAc : hexane eluent), **14** as a white foam (0.24 g, 40 %). ν_{max} (neat)/cm^{-1} 2941 (w), 1745 (s). δ_H (600 MHz, $CDCl_3$) 8.04–8.02 (m, 2H, Ar), 7.57–7.54 (m, 1H, Ar), 7.44–7.41 (m, 2H, Ar), 5.82–5.75 (m, 1H, C*H*=CH_2), 5.30–4.92 (m, 12H, H1, H1′, H2, H2′, H2″, H3′, H3″, H4, H4′, H4″, CH=CH_2), 4.56 (d, *J* 7.8, 1H, H1″), 4.34 (dd, *J* 9.6, 9.6, 1H, H3), 4.31–4.27, 4.17–4.11, 3.97–3.89, 3.86–3.81, 3.72–3.68, 3.55–3.51 (6 m, 9H, H5, H5′, H5″, H6, H6, H6″, H6″, CH_2), 2.11 (s, 3H, C(O)CH_3), 2.09 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.99 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3), 1.93 (s, 3H, C(O)CH_3), 1.77 (s, 3H, C(O)CH_3), 1.15 (d, *J* 6.6, 3H). δ_C (150.8 MHz, $CDCl_3$) 170.8 (C=O), 170.3 (C=O), 170.1 (C=O), 169.9 (C=O), 169.6 (C=O), 169.5 (C=O), 169.4 (C=O), 169.3 (C=O), 165.5 (C=O), 133.4, 133.3 (*C*H=CH_2, Ar), 130.1 (Ar), 129.3 (Ar), 128.5 (Ar), 118.0 (CH=*C*H_2), 101.1, 99.3, 94.7 (C1, C1′, C1″), 77.3, 73.3, 72.8, 72.0, 71.2, 71.0, 70.0, 69.8, 69.0, 68.5, 67.4 (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′, C5″), 68.8, 68.4, 62.0 (C6, C6″, CH_2) 21.1 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 17.5 (CH_3). *m/z* (HR-MS) (ESI) 991.3113; $[M+Na]^+$ requires 991.3059. Anal. Calc. for $C_{44}H_{56}O_{24}$: C 54.54, H 5.83. Found: C 54.42, H 5.95 %.

2,3,4-Tri-O-acetyl-α-L-rhamnosyl-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-α/β-D-glucose **15**

Palladium(II) chloride (80 mg, 0.45 mmol) was added to a solution of the disaccharide **12** (0.29 g, 0.43 mmol) in a solution of CH_3COOH, NaOAc, and H_2O (35 mL, 1.4 g, and 1.9 mL, respectively), and stirred at room temperature for 30 h. After the reaction was complete (as assessed by TLC), the reaction mixture was filtered through celite and concentrated. The residue was suspended in CH_2Cl_2 (50 mL) and washed with water, hydrochloric acid (1M, 3 × 20 mL), water (20 mL), saturated $NaHCO_3$ (2 × 20 mL), and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (2 : 3 EtOAc: hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.22), **15** as a colourless glass (0.22 g, 79 %). ν_{max} (neat)/cm^{-1} 2984 (w), 1747 (s). δ_H (600 MHz, $CDCl_3$) (major anomer) 8.08–8.03 (m, 2H, Ar), 7.60–7.50 (m, 1H, Ar), 7.46–7.40 (m, 2H, Ar), 5.60 (dd, *J* 3.6, 3.6, 1H, H1), 5.24–5.08, 5.03–4.92 (2 m, 6H, H1′, H2, H2′, H3′, H4, H4′), 4.39 (dd, *J* 9.6, 9.6, 1H, H3), 4.28–4.19, 4.17–4.07, 3.92–3.82 (3 m, 4H, H5, H5′, H6, H6) 3.19 (d, *J* 3.6, 1H, OH), 2.12 (s, 3H, C(O)CH_3), 2.11 (s, 6H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3), 1.95 (s, 3H, C(O)CH_3), 1.78 (s, 3H, C(O)CH_3), 1.17 (d, *J* 6.0, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) (major anomer) 171.0 (C=O), 170.1 (C=O), 169.6 (C=O), 169.6 (C=O), 169.4 (C=O), 165.5 (C=O), 133.5 (Ar), 130.1, (Ar), 129.1 (Ar), 128.5 (Ar), 99.3, 90.4 (C1, C1′), 76.8, 73.5, 71.0, 69.9, 69.2, 68.5, 67.8, 67.5 (C2, C2′, C3, C3′, C4, C4′, C5, C5′), 62.3 (C6), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 17.5 (CH_3). *m/z* (HR-MS) (ESI) 679.1653; $[M+K]^+$ requires 679.1640.

2,3,4-Tri-O-acetyl-α-L-rhamnosyl-(1→3)-[2,3,4,6-tetra-O-acetyl-β-D-glucosyl-(1→6)-]-4-O-acetyl-2-O-benzoyl-α/β-D-glucose **16**

Palladium(II) chloride (42 mg, 0.24 mmol) was added to a solution of **14** (0.21 g, 0.22 mmol) in a mixture of CH_3COOH, NaOAc, and H_2O (25 mL, 0.98 g, and 1.3 mL, respectively), and stirred at room temperature for 48 h. After the reaction was complete (as assessed by TLC), the reaction mixture was filtered through celite and concentrated. The residue was suspended in EtOAc (20 mL) and washed with water (2 × 20 mL), saturated $NaHCO_3$ solution (20 mL), and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (EtOAc : hexane = 13 : 7) afforded, after concentration of the appropriate fractions (R_F 0.20, 3 : 2 EtOAc : hexane eluent), **16** as a white foam (0.18 g, 87 %). ν_{max} (neat)/cm^{-1} 3473 (w), 1744 (s). δ_H (600 MHz, $CDCl_3$) (major anomer) 8.00–8.03 (m, 2H, Ar), 7.58–7.53 (m, 1H, Ar), 7.45–7.41 (m, 2H, Ar), 5.55 (dd, *J* 3.6, 3.6, 1H, H1), 5.23–4.91 (m, 9H, H1′, H2, H2′, H2″, H3′, H3″, H4, H4′, H4″), 4.60 (d, *J* 8.4, 1H, H1″), 4.39 (dd, *J* 8.0, 8.0, 1H, H3), 4.24–4.19, 3.89–3.82, 3.73–3.69, 3.62–3.59 (4 m, 7H, H5, H5′, H5″, H6, H6, H6″, H6″), 2.12

(s, 3H, C(O)CH_3), 2.09 (s, 3H, C(O)CH_3), 2.03 (s, 3H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.98 (s, 3H, C(O)CH_3), 1.95 (s, 3H, C(O)CH_3), 1.77 (s, 3H, C(O)CH_3), 1.74 (s, 3H, C(O)CH_3), 1.15 (d, *J* 6.0, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) (major anomer) 170.9 (C=O), 170.4 (C=O), 170.1 (C=O), 170.0 (C=O), 169.9 (C=O), 169.6 (C=O), 169.4 (C=O), 165.5 (C=O), 133.7 (Ar), 133.5 (Ar), 130.1 (Ar), 128.5 (Ar), 101.3, 99.2, 90.1 (C1, C1′, C1″), 76.7, 73.6, 72.7, 71.5, 71.0, 70.1, 69.9, 69.8, 68.7, 68.5, 68.5, 67.4 (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′, C5″), 69.3, 61.9 (C6, C6″), 21.1 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 17.4 (CH_3). *m/z* (HR-MS) (ESI) 951.2750; $[M+Na]^+$ requires 951.2746.

2-(4-Acetoxy-3-methoxyphenyl)ethyl 2,3,4-tri-O-acetyl-α-L-rhamnosyl-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-β-D-glucoside ***18***

Trichloroacetonitrile (65 μL, 0.65 mmol) and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU; 50 μL) were added to a solution of the hemiacetal **15** (0.21 g, 0.33 mmol) in CH_2Cl_2 (5 mL) at 0°C, and the resulting solution was left to stand at room temperature for 15 h. After the reaction was complete (as assessed by TLC), the reaction mixture was concentrated. Flash chromatography of the resulting oil (2 : 3 EtOAc : hexane eluent) afforded a white foam (0.14 g). The foam was then suspended in CH_2Cl_2 (6 mL) and stirred at −30°C with **17**[16] (30 mg, 0.14 mmol) and dry 4-Å molecular sieves (0.25 g) for 30 min. The resulting mixture was cooled to −30°C and treated with TMSOTf (50 μL), and after 5 min, stirred at room temperature for 1 h. After the reaction was complete (as assessed by TLC), the reaction mixture was cooled to 0°C and quenched with triethylamine (100 μL), and filtered through celite and concentrated. Flash chromatography of the resulting oil (1 : 1 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.14), **18** as a colourless glass (89 mg, 33 % over two steps). ν_{max} (neat)/cm^{-1} 2962 (w), 1741 (s). δ_H (600 MHz, $CDCl_3$) 8.00–7.94 (m, 2H, Ar), 7.57–7.51 (m, 1H, Ar), 7.44–7.39 (m, 2H, Ar), 6.72 (d, *J* 1.2, 1H, Ar), 6.65 (d, *J* 7.8, 1H, Ar), 6.62 (dd, *J* 1.8, 7.8, 1H, Ar), 5.30 (dd, *J* 7.8, 9.6, 1H, H2), 5.16 (dd, *J* 9.6, 9.6, 1H, H4), 5.10 (dd, *J* 3.6, 10.2, 1H, H3′), 4.90–4.86 (m, 2H, H2′, H4′), 4.83 (d, *J* 1.8, 1H, H1′), 4.57 (d, *J* 7.8, 1H, H1), 4.24 (dd, *J* 4.8, 12, 1H, H6), 4.14–4.04 (m, 2H, H5, H6), 4.00 (dd, *J* 9.0, 9.0, 1H, H3), 3.84–3.81 (m, 1H, H5′), 3.72 (s, 3H, OCH_3), 3.65–3.60 (m, 2H, CH_2), 2.77 (dd, *J* 6.6, 6.6, 2H, CH_2), 2.25 (s, 3H, C(O)CH_3), 2.09 (s, 6H, C(O)CH_3), 2.08 (s, 3H, C(O)CH_3), 1.97 (s, 3H, C(O)CH_3), 1.78 (s, 3H, C(O)CH_3), 1.13 (d, *J* 6.6, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.9 (C=O), 170.2 (C=O), 169.6 (C=O), 169.5 (C=O), 169.3 (C=O), 164.7 (C=O), 150.8 (Ar), 138.2 (Ar), 137.4 (Ar), 133.3 (Ar), 130.1 (Ar), 129.4 (Ar), 128.4 (Ar), 122.5 (Ar), 121.0 (Ar), 113.3 (Ar), 101.1, 99.2 (C1, C1′), 80.2, 73.0, 72.3, 71.1, 70.6, 70.0, 69.4, 68.4, 67.4, 62.3, 55.9 (C2, C2′, C3, C3′, C4, C4′, C5, C5′, C6, CH_2, OCH_3), 36.1 (CH_2), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 17.5 (CH_3). *m/z* (HR-MS) (ESI) 871.1653; $[M+K]^+$ requires 871.2427.

2-(4-Acetoxy-3-methoxyphenyl)ethyl 2,3,4-tri-O-acetyl-α-L-rhamnosyl-(1→3)-[2,3,4,6-tetra-O-acetyl-β-D-glucosyl-(1→6)-]-4-O-acetyl-2-O-benzoyl-β-D-glucoside ***19***

Trichloroacetonitrile (36 μL, 0.36 mmol) and DBU (0.07 mL) were added to a solution of **16** (0.17 g, 0.18 mmol) in CH_2Cl_2 (4 mL) and the resulting solution was left to stand overnight. The reaction mixture was concentrated. Flash chromatography of the resulting oil (1 : 1EtOAc : hexane eluent) afforded a yellow oil (58 mg). The yellow oil was then suspended in CH_2Cl_2 (3 mL) and stirred with **17**[16] (12 mg, 0.057 mmol) and dry 4-Å molecular sieves (0.10 g) for 15 min. The mixture was then cooled to −30°C and treated with TMSOTf (30 μL). The reaction mixture was stirred at room temperature and once complete (as assessed by TLC, 1 h) was cooled to 0°C and quenched with triethylamine (100 μL), filtered through celite, and concentrated. Flash chromatography of the resulting oil (3 : 2 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.24), **19** as a colourless glass (30 mg, 15 % over two steps). ν_{max} (neat)/cm^{-1} 1751 (s). δ_H (600 MHz, $CDCl_3$) 7.98–7.96 (m, 2H, Ar), 7.56–7.53 (m, 1H, Ar), 7.43–7.40 (m, 2H, Ar), 6.76 (d, *J* 1.8, 1H, Ar), 6.68–6.64 (m, 2H, Ar), 5.25 (dd, *J* 7.8, 9.0, 1H, H2), 5.16 (dd, *J* 9.0, 9.0, 1H, H4), 5.10–5.07, 4.98–4.86 (2 m, 6H, H2′, H2″, H3′, H3″, H4′, H4″), 4.82 (d, *J* 1.8, 1H, H1′), 4.56, 4.52 (2d, 2H, H1, H1″), 4.27 (dd, *J* 4.8, 12.6, 1H, H6), 4.12–4.05, 3.84–3.80, 3.68–3.61 (3 m, 8H, H5, H5′, H5″, H6, H6″, H6″, CH_2), 3.99 (dd, *J* 9.0, 9.0, 1H, H3), 3.75 (s, 3H, OCH_3), 2.77 (t, *J* 6.6, 2H, CH_2), 2.25 (s, 3H, C(O)CH_3), 2.08 (s, 6H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.98 (s, 6H, C(O)CH_3), 1.91 (s, 3H, C(O)CH_3), 1.87 (s, 3H, C(O)CH_3), 1.79 (s, 3H, C(O)CH_3), 1.13 (d, *J* 6.6, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.7 (C=O), 170.3 (C=O), 170.1 (C=O), 169.8 (C=O), 169.6 (C=O), 169.5 (C=O), 169.4 (C=O), 169.3 (C=O), 169.1 (C=O), 164.7 (C=O), 150.8 (Ar), 138.2 (Ar), 137.5 (Ar), 133.3 (Ar), 130.0 (Ar), 129.4 (Ar), 128.4 (Ar), 122.5 (Ar), 121.0 (Ar), 113.4 (Ar), 100.9, 100.7, 99.0 (C1, C1′, C1″), 79.8, 73.9, 73.0, 72.9, 72.0, 71.3, 71.0, 70.2, 69.9, 68.4, 68.3, 67.4, 55.9, (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′, C5″, OCH_3), 70.4, 68.7, 61.9 (C6, C6″, CH_2), 36.0 (CH_2), 21.1 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 20.5 (C(O)*C*H_3), 17.4 (CH_3). *m/z* (HR-MS) (ESI) 1143.3586; $[M+Na]^+$ requires 1143.3533.

2-(4-Hydroxy-3-methoxyphenyl)ethyl α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (Cistanoside E) ***1***

Sodium methoxide in MeOH (0.50 mL, 0.2 M) was added to a solution of **18** (84 mg, 0.10 mmol) in MeOH (5 mL) at 0°C and the resulting solution was stirred at room temperature for 4 days. After the reaction was complete (as assessed by TLC), the reaction mixture was treated with Amberlite IR-120 (H^+) resin (100 mg), filtered, and concentrated. Flash chromatography of the resulting oil (1 : 4 MeOH : $CHCl_3$ eluent) afforded, after concentration of the appropriate fractions (R_F 0.26), **1** as a colourless glass (41 mg, 85 %). ν_{max} (neat)/cm^{-1} 2969 (w), 1736 (s), 1512 (w), 1429 (w), 1368 (w), 1217 (s), 1143 (w), 1029 (s). ^1H and ^{13}C NMR data were consistent with that found in the literature.[6] *m/z* (HR-MS) (ESI) 499.1791; $[M+Na]^+$ requires 499.1791. Anal. Calc. for $C_{21}H_{32}O_{12}$: C 52.94, H 6.77. Found: C 52.78, H 6.84 %.

2-(4-Hydroxy-3-methoxyphenyl)ethyl α-L-rhamnopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)-]-β-D-glucopyranoside (Leonoside F) ***2***

Sodium methoxide (10 mg, 0.19 mmol) was added to a solution of **19** (28 mg, 0.025 mmol) in MeOH (3 mL) and the resulting solution was stirred at room temperature for 5 days. After the reaction was complete (as assessed by TLC), the reaction mixture was treated with Amberlite IR-120 (H^+) resin (100 mg), filtered, and concentrated. Flash chromatography of the resulting oil (39 : 11 : 4 EtOAc : MeOH : H_2O eluent) afforded, after

concentration of the appropriate fractions (R_F 0.32), **2** as a colourless glass (8 mg, 50 %). ν_{max} (neat)/cm^{-1} 3365 (s), 2928 (w), 1585 (w), 1518 (w), 1375 (w), 1272 (w), 1037 (s). ^{1}H and ^{13}C NMR data were consistent with that found in the literature.[6,9] *m/z* (HR-MS) (ESI) 677.2052; $[M+K]^+$ requires 677.2059.

*Allyl 3-O-acetyl-4-O-benzoyl-2-O-chloroacetyl-α-L-rhamnoside **20***

Chloroacetyl chloride (0.45 mL, 5.7 mmol) was added to a solution of **21**[17] (1.4 g, 4.0 mmol) in pyridine (3 mL) and CH_2Cl_2 (15 mL) at 0°C, and the solution was stirred at room temperature for 2 h. After the reaction was complete (as assessed by TLC), the reaction mixture was quenched with MeOH (10 mL) and concentrated. The residue was suspended in EtOAc (50 mL) and washed with hydrochloric acid (1 M, 30 mL), saturated $NaHCO_3$ solution (30 mL), and brine (30 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (1 : 9 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.25), **20** as a white foam (1.1 g, 65 %). ν_{max} (neat)/cm^{-1} 2983 (w), 1753 (s), 1728 (s). δ_H (600 MHz, $CDCl_3$) 8.00–7.99 (m, 2H, Ar), 7.59–7.56 (m, 1H, Ar), 7.46–7.43 (m, 2H, Ar), 5.95–5.88 (m, 1H, C*H*=CH$_2$), 5.55 (dd, *J* 3.6, 10.2, 1H, H3), 5.37–5.29 (m, 3H, H2, H4, CH=C*H*$_2$), 5.27–5.24 (m, 1H, CH=C*H*$_2$), 4.86 (d, *J* 1.2, 1H, H1), 4.26–4.16, 4.08–4.02 (2 m, 5H, H5, CH_2, C(O)CH_2Cl), 1.89 (s, 3H, C(O)CH_3), 1.27 (d, *J* 6.6, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.0 (C=O), 166.9 (C=O), 165.8 (C=O), 133.6, 133.2 (*C*H=CH$_2$, Ar), 129.9 (Ar), 129.4 (Ar), 128.7 (Ar), 118.5 (CH=*C*H$_2$), 96.3 (C1), 72.0, 71.6, 69.0, 68.7, 66.9 (C2, C3, C4, C5, CH_2) 40.9 (C(O)*C*H$_2$Cl), 20.8 (C(O)*C*H$_3$), 17.6 (CH_3). *m/z* (HR-MS) (ESI) 465.0743; $[M+K]^+$ requires 465.0719. Anal. Calc. for $C_{20}H_{23}ClO_8$: C 56.28, H 5.43. Found: C 56.14, H 5.61 %.

*3-O-Acetyl-4-O-benzoyl-2-O-chloroacetyl-α/β-L-rhamnose **22***

Palladium(II) chloride (0.28 g, 1.6 mmol) was added to a solution of **20** (0.64 g, 1.5 mmol) in a mixture of CH_3COOH, NaOAc, and H_2O (70 mL, 2.8 g, and 3.8 mL, respectively), and stirred at room temperature for 20 h. After the reaction was complete (as assessed by TLC), the reaction mixture was filtered through celite and concentrated. The residue was suspended in CH_2Cl_2 (100 mL) and washed with water (50 mL), saturated $NaHCO_3$ solution (3 × 50 mL), and brine (2 × 50 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (3 : 7 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.32), **22** as a white foam (0.53 g, 91 %). ν_{max} (neat)/cm^{-1} 3462 (w), 2985 (w), 1726 (w). δ_H (600 MHz, $CDCl_3$) (major anomer) 8.02–7.97 (m, 2H, Ar), 7.60–7.57 (m, 1H, Ar), 7.46–7.43 (m, 2H, Ar), 5.61 (dd, *J* 3.0, 10.0, 1H, H3), 5.40–5.25 (m, 3H, H1, H2, H4), 4.34–4.15 (m, 3H, H5, C(O)CH_2Cl), 3.42 (d, *J* 3.0, 1H, OH), 1.91 (s, 3H, C(O)CH_3), 1.26 (d, *J* 4.5, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) (major anomer) 170.2 (C=O), 167.0 (C=O), 165.8 (C=O), 133.6 (Ar), 129.9 (Ar), 129.3 (Ar), 128.7 (Ar), 92.0 (C1), 72.4, 71.6, 68.7, 66.8 (C2, C3, C4, C5), 40.9 (C(O)*C*H$_2$Cl), 20.8 (C(O)*C*H$_3$), 17.7 (CH_3). *m/z* (HR-MS) (ESI) 425.0406; $[M+K]^+$ requires 425.0406.

*Allyl 3-O-acetyl-4-O-benzoyl-2-O-chloroacetyl-α-L-rhamnosyl-(1→3)-2-O-benzoyl-4,6-O-benzylidene-α-D-glucoside **23***

Trichloroacetonitrile (0.23 mL, 2.3 mmol) and DBU (0.1 mL) were added to a solution of **22** (0.44 g, 1.1 mmol) in CH_2Cl_2 (5 mL) at 0°C, and the resulting solution was left to stand at room temperature for 15 h. After the reaction was complete (as assessed by TLC), the reaction mixture was concentrated. Flash chromatography of the resulting oil (3 : 17 EtOAc : hexane eluent) afforded a white foam (0.24 g). The foam was then suspended in CH_2Cl_2 (10 mL) and stirred with **6**[11] (0.14 g, 0.34 mmol) and dry 4-Å molecular sieves (0.4 g) for 30 min. The mixture was then cooled to −30°C and treated with TMSOTf (50 µL) and after 5 min, stirred at room temperature for 30 min. After reaction was complete (as assessed by TLC), the reaction mixture was cooled to 0°C, quenched with triethylamine (100 µL), filtered through celite, and concentrated. Flash chromatography of the resulting oil (1 : 3 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.58, 3 : 7 EtOAc : hexane eluent), **23** as a white foam (0.26 g, 30 % over two steps). ν_{max} (neat)/cm^{-1} 3370 (w), 2968 (w), 1751 (w), 1725 (s), 1695 (w). δ_H (600 MHz, $CDCl_3$) 8.03–7.98 (m, 2H, Ar), 7.89–7.85 (m, 2H, Ar), 7.64–7.57 (m, 2H, Ar), 7.53–7.40 (m, 6H, Ar), 7.22–7.13 (m, 3H, Ar), 5.85–5.78 (m, 1H, C*H*=CH$_2$), 5.64 (s, 1H, PhCH), 5.46 (dd, *J* 3.6, 10.2, 1H, H3′), 5.31–5.25 (m, 1H, CH=C*H*$_2$), 5.24 (d, *J* 3.6, 1H, H1), 5.18–5.10 (m, 4H, H2, H2′, H4′, CH=C*H*$_2$), 5.07 (d, *J* 1.8, 1H, H1′), 4.50 (dd, *J* 9.6, 9.6, 1H, H3), 4.37–4.32, 4.06–3.97, 3.85–3.83 (3 m, 6H, H5, H5′, H6, H6, CH_2, C(O)CH_2Cl), 4.24–4.18 (m, 1H, CH_2), 3.91 (A part of an ABq, *J* 15.0, 1H, C(O)CH_2Cl), 3.78 (dd, *J* 9.6, 9.6, 1H, H4), 1.82 (s, 3H, C(O)CH_3), 0.81 (d, *J* 6.6, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.0 (C=O), 166.1 (C=O), 165.8 (C=O), 165.7 (C=O), 137.2 (Ar), 133.7 (Ar), 133.5, 133.4 (Ar, *C*H=CH$_2$), 129.9 (Ar), 129.8 (Ar), 129.4 (Ar), 129.3 (Ar), 129.2 (Ar), 128.7 (Ar), 128.6 (Ar), 128.3 (Ar), 126.4 (Ar), 118.0 (CH=*C*H$_2$), 102.2, 97.8, 96.2 (C1, C1′, PhCH), 79.7, 74.7, 73.5, 71.5, 71.4, 69.1, 69.0, 68.8, 66.7, 63.2 (C2, C2′, C3, C3′, C4, C4′, C5, C5′, C6, CH_2), 40.5 (C(O)*C*H$_2$Cl), 20.7 (C(O)*C*H$_3$), 16.8 (CH_3). *m/z* (HR-MS) (ESI) 781.2246; $[M+H]^+$ requires 781.2263.

*Allyl 3-O-acetyl-4-O-benzoyl-α-L-rhamnosyl-(1→3)-2-O-benzoyl-4,6-O-benzylidene-α-D-glucoside **24***

Thiourea (0.27 g, 3.5 mmol) and 2,6-lutidine (46 µL, 0.40 mmol) were added to a solution of **23** (0.28 g, 0.36 mmol) in MeOH/CH_2Cl_2 (1 : 1, 20 mL), and the solution was stirred at 35°C for 24 h. After the reaction was complete (as assessed by TLC), the reaction mixture was concentrated. The residue was suspended in CH_2Cl_2 (30 mL) and washed with water (15 mL), saturated $NaHCO_3$ (2 × 15 mL), water (15 mL), and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (7 : 13 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.23), **24** as a colourless oil (0.19 g, 74 %). ν_{max} (neat)/cm^{-1} 3420 (w), 2929 (w), 1724 (s). δ_H (600 MHz, $CDCl_3$) 8.07–8.04 (m, 2H, Ar), 7.89–7.85 (m, 2H, Ar), 7.62–7.55 (m, 2H, Ar), 7.52–7.40 (m, 6H, Ar), 7.20–7.15 (m, 3H, Ar), 5.85–5.78 (m, 1H, C*H*=CH$_2$), 5.64 (s, 1H, PhCH), 5.38 (dd, *J* 3.0, 9.6, 1H, H3′), 5.29–5.23 (m, 2H, H1, CH=C*H*$_2$), 5.20 (dd, *J* 10.2, 10.2, 1H, H4′), 5.16–5.12 (m, 2H, H1′, CH=C*H*$_2$), 5.10 (dd, *J* 3.6, 9.6, 1H, H2), 4.53 (dd, *J* 9.6, 9.6 1H, H3), 4.38–4.32, 4.17–3.98 (2 m, 4H, H5, H5′, H6, CH_2), 4.23–4.18 (m, 1H, CH_2), 3.90–3.87 (m, 1H, H2′), 3.83 (t, *J* 10.2, 10.2, 1H, H6), 3.76 (dd, *J* 9.6,9.6 1H, H4), 2.00 (d, *J* 4.2, 1H, OH), 1.90 (s, 3H, C(O)CH_3), 0.81 (d, *J* 6.6, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.0 (C=O), 165.9 (C=O), 165.8 (C=O), 137.2 (Ar), 133.8 (Ar), 133.5, 133.4 (Ar, *C*H=CH$_2$), 129.9 (Ar), 129.8 (Ar), 129.6 (Ar), 129.3 (Ar), 128.9 (Ar), 128.6 (Ar), 128.3 (Ar), 126.4 (Ar), 118.0 (CH=*C*H$_2$), 102.2, 100.0, 96.1 (C1, C1′, PhCH), 79.9, 75.1, 73.3, 72.0, 71.5, 69.8, 66.4, 63.1 (C2, C2′, C3, C3′, C4, C4′, C5,

C5′), 69.1, 69.0 (C6, CH_2), 21.0 (C(O)CH_3), 16.9 (CH_3). *m/z* (HR-MS) (ESI) 743.2107; $[M+K]^+$ requires 743.2106. Anal. Calc. for $C_{38}H_{40}O_{13}$: C 64.76, H 5.72. Found: C 64.55, H 5.79 %.

*Allyl 2,3,4-tri-*O*-acetyl-α-L-arabinosyl-(1→2)-3-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside* ***26***

A mixture of **24** (0.18 g, 0.26 mmol), **25**[19] (0.29 g, 0.69 mmol), and dry 4-Å molecular sieves (0.4 g) in CH_2Cl_2 (10 mL) were stirred at room temperature for 15 min. The reaction mixture was cooled to −30°C and treated with TMSOTf (50 µL) and after 5 min, the mixture was stirred at room temperature for 1 h. After the reaction was complete (as assessed by TLC), the reaction mixture was cooled to 0°C and treated with triethylamine (100 µL), filtered through celite, and concentrated. Flash chromatography of the resulting oil (2 : 3 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.33), **26** as a white foam (0.12 g, 46 %). ν_{max} (neat)/cm^{-1} 2940 (w), 1748 (s). δ_H (600 MHz, $CDCl_3$) 8.11–8.05 (m, 2H, Ar), 7.83–7.80 (m, 2H, Ar), 7.58–7.38 (m, 8H, Ar), 7.20–7.12 (m, 3H, Ar), 5.82–5.24 (m, 1H, C*H*=CH_2), 5.64 (s, 1H, PhCH), 5.31 (dd, *J* 3.6, 10.2, 1H, H3′), 5.27–5.21 (m, 2H, H1′, CH=CH_2), 5.18 (d, *J* 4.2, 1H, H1), 5.15–5.05 (m, 4H, H2, H2″, H4′, CH=CH_2), 4.97–4.94 (m, 1H, H4″), 4.85 (dd, *J* 3.6, 9.6, 1H, H3″), 4.52 (dd, *J* 9.6, 9.6, 1H, H3), 4.34–4.32 (m, 2H, H5′, H6), 4.21–4.16 (m, 1H, CH_2), 4.11 (d, *J* 7.2, 1H, H1″), 4.06–3.99 (m, 1H, H5), 3.99–3.94 (m, 1H, CH_2), 3.90–3.87 (m, 1H, H2′), 3.82 (dd, *J* 10.2, 10.2, 1H, H6), 3.76 (dd, *J* 9.6, 9.6, 1H, H4), 2.94 (dd, *J* 1.8, 12.6, 1H, H5″), 2.70–2.60 (m, 1H, H5″), 2.15 (s, 3H, C(O)CH_3), 2.14 (s, 3H, C(O)CH_3), 2.02 (s, 3H, C(O)CH_3), 1.90 (s, 3H, C(O)CH_3), 0.81 (d, *J* 6.0, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.4 (C=O), 170.2 (C=O), 169.7 (C=O), 165.8 (C=O), 165.3 (C=O), 137.2 (Ar), 133.4, 133.4, 133.2 (Ar, *C*H=CH_2) 130.2 (Ar), 129.8 (Ar), 129.7 (Ar), 129.3 (Ar), 129.3 (Ar), 128.6 (Ar), 128.5 (Ar), 128.2 (Ar), 126.4 (Ar), 117.8 (CH=*C*H_2), 102.4, 102.2, 99.2, 96.0 (C1, C1′, C1″, PhCH), 79.9, 77.0, 74.9, 72.4, 71.9, 71.1, 69.8, 68.8, 67.4, 66.3, 63.0 (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′), 69.1, 68.9, 62.2 (C5″, C6, CH_2), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 16.9 (CH_3). *m/z* (HR-MS) (ESI) 985.3155; $[M+Na]^+$ requires 985.3106.

*Allyl 2,3,4-tri-*O*-acetyl-α-L-arabinosyl-(1→2)-3-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-glucoside* ***27***

Allyl 2,3,4-tri-*O*-acetyl-α-L-arabinosyl-(1→2)-3-*O*-acetyl-4-*O*-benzoyl-β-L-rhamnosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucoside **26** (0.10 g, 0.10 mmol) was treated with CH_3COOH/H_2O (4 : 1, 5 mL) and stirred at 80°C for 6 h. After the reaction was complete (as assessed by TLC), the reaction mixture was concentrated and the residue was co-evaporated with toluene (3 × 3 mL). The residue was suspended in pyridine (5 mL) and the resulting mixture was treated with acetic anhydride (0.10 mL, 1.1 mmol) and DMAP (25 mg), and stirred at room temperature for 12 h. After the reaction was complete (as assessed by TLC), the reaction mixture was quenched with MeOH (5 mL) and concentrated. The residue was suspended in EtOAc (15 mL) and washed with water (10 mL), hydrochloric acid (1 M, 3 × 5 mL), water (10 mL), saturated $NaHCO_3$ solution (10 mL), and brine (10 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (2 : 3 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.22), **27** as a colourless glass (79 mg, 82 %). ν_{max} (neat)/cm^{-1} 2939 (w), 1735 (s). δ_H (600 MHz, $CDCl_3$) 8.10–8.05 (m, 2H, Ar), 7.96–7.92 (m, 2H, Ar), 7.59–7.53 (m, 2H, Ar), 7.46–7.40 (m, 4H, Ar), 5.85–5.75 (m, 1H, C*H*=CH_2), 5.29–5.10 (m, 9H, H1, H1′, H2, H2″, H3′, H4, H4′, CH=CH_2), 5.07–5.03 (m, 1H, H4″), 4.90–4.85 (m, 1H, H3″), 4.40 (dd, *J* 9.6, 9.6, 1H, H3), 4.27 (dd, *J* 2.4, 4.2, 1H, H6), 4.19–4.08, 4.03–3.92, (2 m, 5H, H5, H5′, H6, CH_2), 4.07, (d, *J* 7.2, 1H, H1″), 3.80–3.78 (m, 1H, H2′), 3.20–3.07 (m, 2H, H5″, H5″), 2.14 (s, 3H, C(O)CH_3), 2.13 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.12 (s, 3H, C(O)CH_3), 2.04 (s, 3H, C(O)CH_3), 1.81 (s, 3H, C(O)CH_3), 1.20 (d, *J* 6.0, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) 170.9 (C=O), 170.4 (C=O), 170.2 (C=O), 169.7 (C=O), 169.6 (C=O), 169.6 (C=O), 169.5 (C=O), 169.4 (C=O), 133.4, 133.4, 133.3 (Ar, *C*H=CH_2), 130.2 (Ar), 129.8 (Ar), 129.6 (Ar), 129.4 (Ar), 128.6 (Ar), 128.5 (Ar), 118.2 (CH=*C*H_2), 102.4, 100.3, 95.3 (C1, C1′, C1″), 77.0, 76.2, 73.5, 71.7, 70.5, 69.8, 69.2, 69.0, 68.0, 67.4, 67.7, (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′), 68.8, 62.5, 62.2 (C5″, C6, CH_2), 21.2 (C(O)*C*H_3), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 17.7 (CH_3). *m/z* (HR-MS) (ESI) 997.2706; $[M+K]^+$ requires 997.2744. Anal. Calc. for $C_{46}H_{54}O_{22}$: C 57.62, H 5.68. Found: C 57.49, H 5.78 %.

*2,3,4-tri-*O*-acetyl-α-L-arabinosyl-(1→2)-3-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α/β-D-glucose* ***28***

Palladium(II) chloride (15 mg, 0.085 mmol) was added to a solution of **27** (75 mg, 0.078 mmol) in a mixture of CH_3COOH, NaOAc, and H_2O (17 mL, 0.7 g, and 0.9 mL, respectively), and stirred at room temperature for 48 h. After the reaction was complete (as assessed by TLC), the reaction mixture was filtered through celite and concentrated. The residue was suspended in CH_2Cl_2 (20 mL) and washed with water (2 × 10 mL), hydrochloric acid (1 M, 10 mL), water (2 × 10 mL), saturated $NaHCO_3$ solution (3 × 15 mL), and brine (15 mL), dried over $MgSO_4$, filtered, and concentrated. Flash chromatography of the resulting oil (1 : 1 EtOAc : hexane eluent) afforded, after concentration of the appropriate fractions (R_F 0.20), **28** as a colourless glass (58 mg, 81 %). ν_{max} (neat)/cm^{-1} 2971 (w), 1735 (s). δ_H (600 MHz, $CDCl_3$) (major anomer) 8.07–8.03 (m, 2H, Ar), 7.93–7.91 (m, 2H, Ar), 7.56–7.51 (m, 2H, Ar), 7.44–7.39 (m, 4H, Ar), 5.53–5.51 (m, 1H, H1), 5.25–5.08 (m, 6H, H1′, H2, H2″, H3′, H4, H4′), 5.05–5.02 (m, 1H, H4″), 4.87–4.85 (m, 1H, H3″), 4.43 (dd, *J* 9.6, 9.6, 1H, H3), 4.24–4.21, 4.12–4.05, 3.95–3.92, 3.79–3.77 (4 m, 6H, H1″, H2′, H5, H5′, H6, H6), 3.18–3.05 (m, 2H, H5″, H5″), 2.12 (s, 3H, C(O)CH_3), 2.11 (s, 6H, C(O)CH_3), 2.10, (s, 3H, C(O)CH_3), 2.01 (s, 3H, C(O)CH_3), 1.79 (s, 3H, C(O)CH_3), 1.18 (d, *J* 6.6, 3H, CH_3). δ_C (150.8 MHz, $CDCl_3$) (major anomer) 171.0 (C=O), 170.3 (C=O), 170.2 (C=O), 169.7 (C=O), 169.6 (C=O), 169.5 (C=O), 165.4 (C=O), 165.4 (C=O), 133.4 (Ar), 133.3 (Ar), 130.2 (Ar), 129.8 (Ar), 129.6 (Ar), 129.4 (Ar), 128.7 (Ar), 128.6 (Ar), 102.3, 100.2, 90.2, (C1, C1′, C1″) 77.1, 75.7, 73.8, 71.7, 70.4, 69.8, 69.2, 68.9, 67.7, 67.4, 67.3, 62.6, 62.1 (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′, C5″, C6), 21.0 (C(O)*C*H_3), 20.9 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.8 (C(O)*C*H_3), 20.7 (C(O)*C*H_3), 17.5 (CH_3). *m/z* (HR-MS) (ESI) 941.2698; $[M+Na]^+$ requires 941.2691.

2-(4-Hydroxy-3-methoxyphenyl)ethyl α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (Leonoside E) ***3***

Trichloroacetonitrile (12 µL, 0.12 mmol) and DBU (50 µL) were added to a solution of **28** (53 mg, 0.058 mmol) in CH_2Cl_2 (2.5 mL) at 0°C, and the resulting solution was stirred at room

temperature for 15 h. After the reaction was complete (as assessed by TLC), the reaction mixture was concentrated. Flash chromatography of the resulting oil (1 : 1 EtOAc : hexane eluent) afforded a white foam (14 mg). The foam was then suspended in CH_2Cl_2 (2 mL) and stirred with **17**[16] (45 mg, 0.02 mmol) and dry 4-Å molecular sieves (0.1 g) for 30 min. The mixture was then cooled to −30°C and treated with TMSOTf (50 μL) and after 5 min, stirred at room temperature for 1 h. After the reaction was complete (as assessed by TLC), the reaction mixture was cooled to 0°C and quenched with triethylamine (100 μL), filtered through celite, and concentrated. Flash chromatography of the resulting oil (1 : 1 EtOAc : hexane eluent) afforded **29** a mixture with **17** (13 mg). Sodium methoxide in MeOH (0.50 mL, 0.2 M) was added to the mixture (12 mg) in MeOH (2 mL) at 0°C, and the mixture was allowed to warm to room temperature for 4 days. After the reaction was complete (as assessed by TLC), the reaction mixture was treated with Amberlite IR-120 (H^+) resin (100 mg), filtered, and concentrated. Flash chromatography of the resulting oil (11 : 4 : 35 MeOH : H_2O : EtOAc eluent) afforded, after concentration of the appropriate fractions (R_F 0.20), **3** as a colourless glass (3.5 mg, 10 % over three steps). ν_{max} (neat)/cm^{-1} 3366 (s), 2926 (w), 1639 (s), 1517 (w), 1063 (w). ^{1}H and ^{13}C NMR data were consistent with that found in the literature.[6,9] *m/z* (HR-MS) (ESI) 631.2206; $[M+Na]^+$ requires 631.2214.

Intracellular Reactive Oxygen Species (ROS) Cell Study

Intracellular accumulation of ROS was determined using a 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) assay (Cell Biolabs). HepG2 cells were seeded in 96-well black, clear-bottomed plates at a density of 10^4 cells per well and were grown for 24 h before incubation with 1× DCFH-DA for 30 min at 37°C (according to the manufacturer's instructions), followed by treatment with compounds **1–3** or α-tocopherol. H_2O_2 (250 μM, final concentration) was then added and the cells were incubated at 37°C for 60 min and 3 h. The cells were then washed three times with Hank's balanced salt solution (without phenol red) at room temperature. Cell fluorescence was measured at excitation and emission wavelengths of 480 and 530 nm, respectively. The results are expressed as relative fluorescence units per cell (RFU cell^{-1}).

Determination of Catalase Activity

An OxiSelect Catalase Activity assay kit (Cell Biolabs) was used to determine catalase activity in HepG2 cells. Cells (10^4 cells per well) were grown for 24 h and pre-incubated with **2** or α-tocopherol for 30 min. H_2O_2 (250 μM, final concentration) was then added and the cells were incubated at 37°C for 1 and 3 h. The cells were collected by treating them with cell dissociation buffer (enzyme free). Cell pellets were homogenized in ice-cold PBS and 1 mM EDTA and centrifuged at 10621*g* for 15 min at 4°C. The catalase assay was then performed on 20-μL aliquots of the supernatant according to the manufacturer's instructions and catalase activity was determined by comparison against a catalase standard curve. Results are expressed relative to protein concentration.

Supplementary Material

^{1}H and ^{13}C NMR spectra of the prepared compounds are available on the Journal's website.

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References

[1] D. J. Newman, G. M. Cragg, K. M. Snader, *J. Nat. Prod.* **2003**, *66*, 1022. doi:10.1021/NP030096L
[2] R. Graziose, M. A. Lila, I. Raskin, *Curr. Drug Discov. Technol.* **2010**, *7*, 2. doi:10.2174/157016310791162767
[3] B. K. H. Tan, J. Vanitha, *Curr. Med. Chem.* **2004**, *11*, 1423. doi:10.2174/0929867043365161
[4] S. O. Oyedemi, M. T. Yakubu, A. J. Afolayan, *J. Med. Plant. Res* **2011**, *5*, 119.
[5] X. H. Liu, L. L. Pan, Y. Z. Zhu, *Clin. Exp. Pharmacol. P.* **2012**, *39*, 274. doi:10.1111/J.1440-1681.2011.05630.X
[6] Y. Li, Z. Chen, Z. Feng, Y. Yang, J. Jiang, P. Zhang, *Carbohyd. Res.* **2012**, *348*, 42. doi:10.1016/J.CARRES.2011.10.034
[7] H. I. Duynstee, M. C. de Koning, H. Ovaa, G. A. van der Marel, J. H. van Boom, *Eur. J. Org. Chem.* **1999**, 2623. doi:10.1002/(SICI)1099-0690(199910)1999:10<2623::AID-EJOC2623>3.0.CO;2-K
[8] H. Kobayashi, H. Karasawa, T. Miyase, S. Fukushima, *Chem. Pharm. Bull.* **1985**, *33*, 1452. doi:10.1248/CPB.33.1452
[9] G. Gu, Y. Zhao, Z. Guo, *Carbohyd. Res.* **2013**, *380*, 174. doi:10.1016/J.CARRES.2013.07.012
[10] M. Ota, K. Takahashi, H. Kofujita, *J. Wood Sci.* **1998**, *44*, 320. doi:10.1007/BF00581314
[11] M. Izumi, K. Fukase, S. Kusumoto, *Biosci. Biotech. Bioch.* **2002**, *66*, 211. doi:10.1271/BBB.66.211
[12] G. Huang, X. Mei, M. Liu, *Carbohydr. Res.* **2005**, *340*, 603. doi:10.1016/J.CARRES.2005.01.015
[13] M. G. Donahue, J. N. Johnston, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5602. doi:10.1016/J.BMCL.2006.08.024
[14] R. R. Schmidt, J. Michel, M. Roos, *Liebigs Ann. Chem.* **1984**, 1343. doi:10.1002/JLAC.198419840710
[15] L. Ban, M. Mrksich, *Angew. Chem. Int. Ed.* **2008**, *47*, 3396. doi:10.1002/ANIE.200704998
[16] S. G. Mills, A. Ali, C. Smith, *Prodrugs of Oxolidinone CETP inhibitors* **2010**, WO2010/039474.
[17] J. Zhang, F. Kong, *Tetrahedron* **2003**, *59*, 1429. doi:10.1016/S0040-4020(03)00075-9
[18] R. K. Jain, C. F. Piskorz, B. Huang, R. D. Locke, H. Han, A. Koenig, A. Varki, L. Khushi, *Glycobiology* **1998**, *8*, 707. doi:10.1093/GLYCOB/8.7.707
[19] T. Murakami, H. Matsuda, M. Inadzuki, K. Hirano, M. Yoshikawa, *Chem. Pharm. Bull.* **1999**, *47*, 1717. doi:10.1248/CPB.47.1717
[20] M. J. Czaja, *Semin. Liver Dis.* **2007**, *27*, 378. doi:10.1055/S-2007-991514
[21] B. Halliwell, *Lancet* **1994**, *344*, 721. doi:10.1016/S0140-6736(94)92211-X
[22] H. E. Poulsen, H. Prieme, S. Loft, *Eur. J. Cancer Prev.* **1998**, *7*, 9.
[23] O. Akyol, H. Herken, E. Uz, E. Fadillioglu, S. Unal, S. Sogut, H. Ozyurt, H. A. Savas, *Prog. Neuro.-Psychoph* **2002**, *26*, 995. doi:10.1016/S0278-5846(02)00220-8
[24] M. J. Czaja, *Antioxid. Redox Sign.* **2002**, *4*, 759. doi:10.1089/152308602760598909
[25] X. Chen, Z. Zhong, Z. Xu, L. Chen, Y. Wang, *Pharmacol. Rep.* **2011**, *63*, 724.
[26] G. E. Henry, R. A. Momin, M. G. Nair, D. L. Dewitt, *J. Agric. Food Chem.* **2002**, *50*, 2231. doi:10.1021/JF0114381
[27] Z. Jia, H. Zhu, J. Li, X. Wang, H. Misra, Y. Li, *Spinal Cord* **2012**, *50*, 264. doi:10.1038/SC.2011.111