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A facile synthesis of isoflavone 7-*O*-glucuronides

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Abstract—An efficient method is presented for the synthesis of isoflavone 7-glucuronides using a *N*-(4-methoxyphenyl)-trifluoroacetimidate glucuronsyl donor. A 4-hexanoyl derivative of the isoflavone is used in the coupling reaction, both for protection and to improve solubility. These glucuronides are the human metabolites of estrogenic dietary isoflavones, but their biological activity and pharmacokinetics have yet to be established as until now there were no good methods for their synthesis.

Isoflavone phytoestrogens, such as daidzein, genistein and glycitein, are a group of polyphenolic compounds with weak estrogenic and antiestrogenic activity, which are present in the human diet,[1,2] in particularly soybeans, soy derived products and chickpeas.[3,4] Epidemiological studies have shown that the consumption of an isoflavone rich diet is associated with a decrease in the incidence of hormone related cancers, for example, breast and prostate cancer.[5,6] Furthermore, it has been suggested that isoflavones may possess other health promoting activities, including chemoprevention of osteoporosis[7] and cardiovascular disease[8] and lessening of menopausal symptoms.[7] However, although many studies have been carried out, there are still questions to be answered concerning the absorption, metabolism and bioavailability of isoflavonoids.[9,10] Daidzein, genistein and glycitein are commonly present in plants and soybeans in the form of glycosides,[11,12] which are hydrolysed by intestinal glucosidases to the aglycones following ingestion.[10,13,14] The isoflavones can then be absorbed or metabolised to other biologically active metabolites including, equol, *O*-demethylangolensin (ODMA) and 6-hydroxy-ODMA.[10,13–15] The aglycones and their metabolites are also transported to the liver, where they undergo conjugation via hepatic enzymes to give *O*-glucuronides and, to a lesser extent, *O*-sulfates.[16,17] In order for studies to progress further there is an urgent need for development of an efficient synthetic route towards the synthesis of pure standards of isoflavone *O*-glucuronides to allow their accurate quantification in biological fluids and to study their possible biological role in vivo. Recently, in our laboratory, Fairley et al. presented an efficient synthesis for isoflavone sulfates,[18] and now we report herein, a new, sophisticated and high yielding procedure for synthesis of isoflavone 7-*O*-glucuronides.

The *O*-glycosylation of polyhydroxyisoflavones utilising classical procedures described in the literature proved to be low yielding or not reproducible (<30%).[19,20] Attempts to synthesise daidzein 7-*O*-glycoside employing a Lewis acid promoted phenyl selenoglycoside or glycosyl phosphates as glycosyl donors also failed to produce a reliable method. Needs and Williamson reported the first synthesis of daidzein-7-yl-β-D-glucopyranosiduronic acid in low yields employing methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide as the glycosyl donor.[21] Recently, Li et al. reported an efficient and facile synthesis of flavonoid 7-*O*-glycosides employing benzoyl protected glycosyl trifluoroacetimidate donors.[22] The method was based on the Lewis acid activated coupling of glycosyl trifluoroacetimidates with the 7-hydroxyl of flavonoid esters. The procedure has now been successfully refined for the synthesis of isoflavon-7-yl-β-D-glucopyranosiduronic acids.

Firstly, *N*-4-methoxyphenyltrifluoroacetimidoyl chloride **2** was prepared by the reaction of trifluoroacetic acid with 4-methoxyaniline and a PPh_3–Et_3N–CCl_4 system.[23] The glycosyl trifluoroacetimidate donor, methyl 2,3,4-triacetyl-D-glucopyranosiduronyl 1-(*N*-4-methoxyphenyl)-2,2,2-trifluoroacetimidate **3**, was then obtained as a mixture of α/ß anomers (1.22:1) in excellent yield

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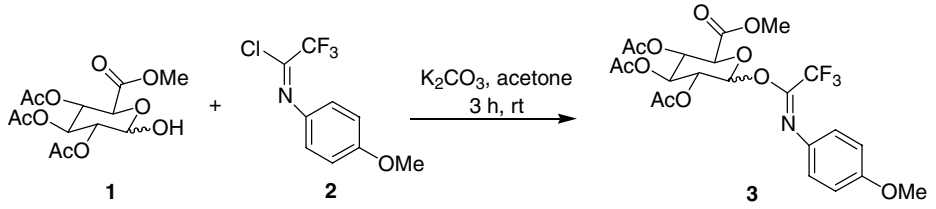
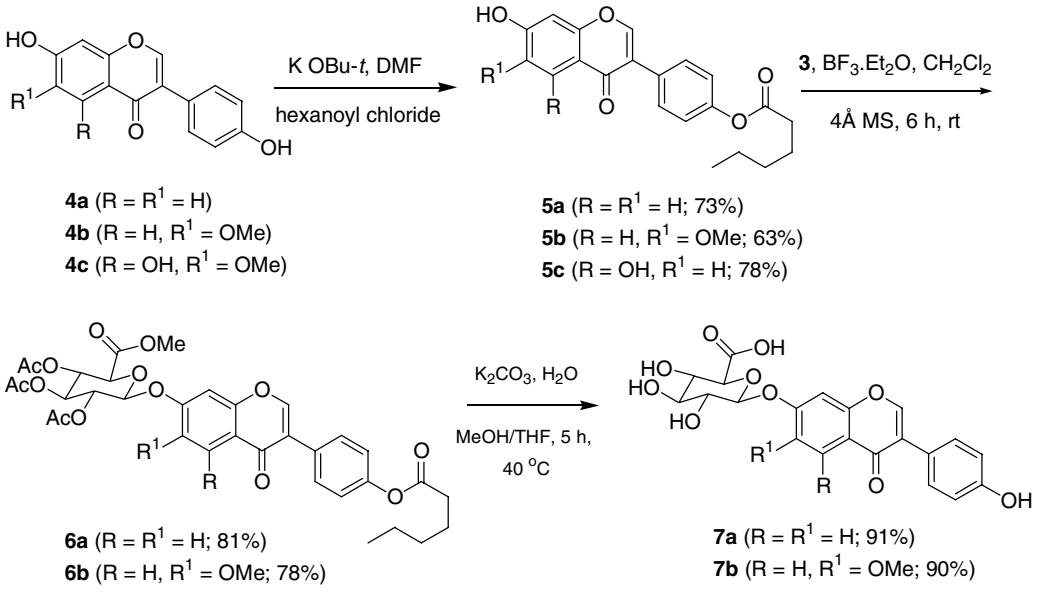
via treatment of methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuranate **1** with **2** (2 equiv) in the presence of K_2CO_3 (2 equiv) in acetone under nitrogen at room temperature for 3 h (Scheme 1). Moisture in the solvent is important, probably as a result of the increased solubility of K_2CO_3. The glucuronyl trifluoroacetimidate **3** was not stable for long periods at room temperature and was therefore kept at −78 °C. The free isoflavones, daidzein **4a**, glycitein **4b** and gensitein **4c** possess more than one hydroxyl group and are poorly soluble in CH_2Cl_2, the solvent for the desired coupling reaction. To circumvent the regioselectivity problem previously encountered using other protocols and to increase the solubility of isoflavones in CH_2Cl_2, we sought to introduce a hexanoyl group at the 4′-hydroxyl group. Regioselective differentiation between the 7- and 4′-hydroxyl groups was accomplished utilising the acidity difference, caused by the electron-withdrawing carbonyl group in ring C. Selective synthesis of the required 4-*O*-hexanoylisoflavones **5a**–**c** was achieved in high yields due to the greater nucleophilicity of the 4′-hydroxyl group via treatment of the 7,4′-diphenolates, generated by reaction of the isoflavone aglycone with an excess of KOBu-*t* (2.2 equiv), with hexanoyl chloride (1 equiv) at 35 °C under argon (Scheme 2). The electron-withdrawing carbonyl group *para* to the phenolate anion at the 7-position significantly diminishes its nucleophilicity and acylation of the 4′-position is favoured.

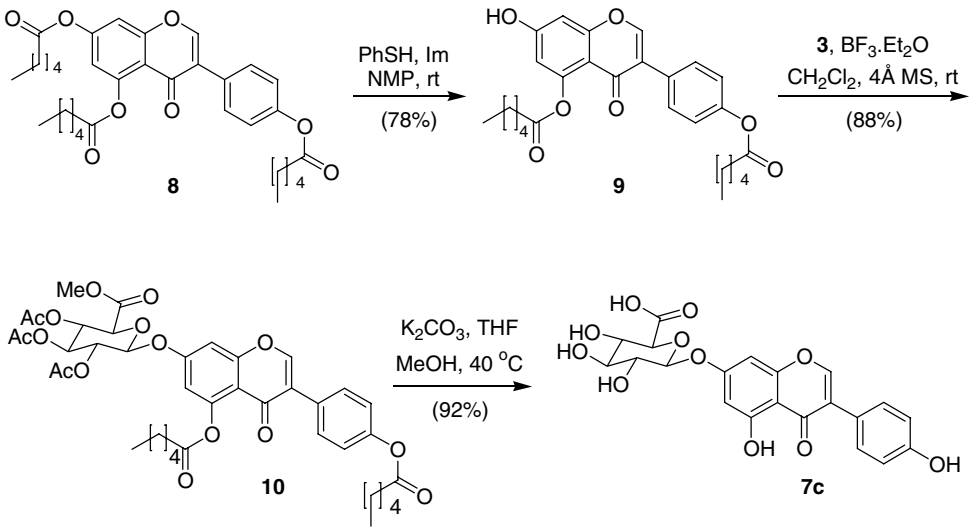
Once the 4′-hexanoylated isoflavones were available, attention was focused on the coupling reaction between the 7-OH of the isoflavone esters and the *O*-acetyl glucuronyl (*N*-*p*-methoxyphenyl)-trifluoroacetimidate **3**. Treatment of 4′-*O*-hexanoyl-daidzein **5a** and glycitein **5b** with *O*-acetyl glucuronyl trifluoroacetimidate **3** in CH_2Cl_2 under the promotion of $BF_3{\cdot}Et_2O$ (0.2 equiv) at room temperature led to the desired coupling products, **6a**[24] and **6b** in 81% and 78% yields, respectively (Scheme 2). However, glucuronidation of 4′-*O*-hexanoyl genistein **5c** with **3** employing the same conditions gave an intractable mixture. Therefore, it was concluded that both the 5- and 4′-hydroxyl groups in genistein **4c** had to be protected. This goal was achieved by regioselective removal of the 7-*O*-acyl group from a peracylated genistein (Scheme 3). Treatment of a solution of genistein **4c** in pyridine with hexanoyl chloride (3.3 equiv) in the presence of DMAP afforded 5,7,4′-tri-*O*-hexanoyl genistein **8** in 91% yield. The 7-*O*-hexanoyl group on the perhexanoylated genistein **8**, which is *para* to the electron-withdrawing carbonyl group, is the most electrophilic ester. Taking advantage of this fact, regioselective release of 7-OH was accomplished via treatment of a solution of **8** in NMP with PhSH (1.2 equiv) in the presence of imidazole to give **9** in 78% yield (Scheme 3). Glucuronidation of **9** with **3** employing the standard conditions afforded **10** in 88% yield. Final removal of the acyl groups to provide the 7-*O*-glucuronides of



Scheme 1.



Scheme 2.



Scheme 3.

daidzein[25] **7a**, glycitein **7b** and genistein **7c** in 90–92% yields, was affected under the action of K_2CO_3 in MeOH:THF:H_2O (5:5:0.5).

In conclusion, we have developed an efficient, high yielding synthesis of isoflavone 7-glucuronides using a novel *N*-(4-methoxyphenyl)-trifluoroacetimidate glucuronsyl donor. The 4-hexanoyl derivatives of daidzein and glycitein were used in the coupling reaction, both for protection and to improve solubility, while for genistein the hydroxyl group at the 5-position also had to be protected. These compounds are now being used to investigate the biological activity and pharmacokinetics of these important human metabolites of dietary isoflavones.

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23. All new compounds gave satisfactory spectroscopic and microanalytical data.
24. Methyl (4′-*O*-hexanoyldaidzein-7-yl-β-D-2″-3″,4″-tri-*O*-acetylglucopyranosid)urinate **6a**: A suspension of 4′-*O*-hexanoyldaidzein **5a** (0.1 g, 0.28 mmol), glucuronyl trifluoroacetamidate **3** (0.30 g, 0.57 mmol) and 4 Å molecular sieves (0.3 g) in dry CH_2Cl_2 (5 mL) was stirred under N_2 at rt. $BF_3{\cdot}Et_2O$ (10 μL, 85 μmol) was added, once the colour of the suspension had turned to orange. After 6 h of stirring at rt, the reaction mixture was quenched with a drop of Et_3N. After filtration, the solvent was evaporated under reduced pressure, and the orange oily residue was subjected to silica gel chromatography (ethyl acetate/petroleum ether 1:1) furnishing **6a** as a white crystalline solid (0.149 g, 81%), mp 193–194 °C; $[\alpha]_D^{20}$ −24.2

(0.001 g mL^{-1} in $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) 0.94 (3H, t, $J = 6.9$ Hz, CH_2CH_3), 1.37–1.42 (4H, m, $CH_2CH_2CH_3$), 1.72–1.82 (2H, m, CH_2CH_2), 2.04, 2.06, 2.08 ($3 \times 3H$, 3s, $3 \times COCH_3$), 2.57 (2H, t, $J = 7.5$ Hz, $O_2CCH_2CH_2$), 3.73 (3H, s, CO_2CH_3), 4.28 (1H, d, $J = 9$ Hz, H-5″), 5.31–5.40 (4H, m, H-1″, H-2″, H-3″, H-4″), 7.04 (1H, d, $J = 2.4$ Hz, H-8), 7.06 (1H, dd, $J = 9.3$, 2.4 Hz, H-6), 7.15 (2H, d, $J = 9.0$ Hz, H-3′, 5′), 7.57 (2H, d, $J = 9.0$ Hz, H-2′, 6′), 7.97 (1H, s, H-2), 7.25 (1H, d, $J = 9.3$ Hz, H-5); MS (EI) m/z 691 (M+Na, 87%), 669 ($M+H^+$, 14%), 357 (100%); Anal. Calcd for $C_{34}H_{36}O_{14}$: C, 61.07; H, 5.43. Found C, 60.70; H, 5.58.

25. Daidzein-7-yl-β-D-glucopyranosiduronic acid **7a**: A solution of K_2CO_3 (0.15 g, 1.09 mmol) in H_2O (0.5 mL) was added to a solution of the ester **6a** (0.25 g, 0.374 mmol) in a mixture of MeOH/THF (6 mL, 1:1) at rt under a nitrogen atmosphere. After stirring for 5 h at 40 °C, the mixture was cooled to rt, neutralised with Dowex-50 H^+ and then filtered and concentrated. The yellow oily residue was purified by preparative HPLC (H_2O:CH_3CN 65:35) furnishing **7a** as a white crystalline solid (0.146 g, 91%), mp 256–257 °C. ^1H NMR (500 MHz, D_2O) 3.48–3.55 (3H, m, H-2″, H-3″, H-4″), 3.76 (1H, d, $J = 9.0$ Hz, H-5″), 4.86 (1H, d, $J = 7.5$ Hz, H-1″), 6.75 (2H, d, $J = 8.5$ Hz, H-3′, 5′), 6.90 (1H, d, $J = 2.5$ Hz, H-8), 6.94 (1H, dd, $J = 9.0$, 2.5 Hz, H-6), 7.12 (2H, d, $J = 8.5$ Hz, H-2′, 6′), 7.72 (1H, d, $J = 9.0$ Hz, H-5), 7.86 (1H, s, H-2); ^{13}C NMR (125 MHz, D_2O) 71.5 (C-4″), 72.4 (C-2″), 75.0 (C-3″), 76.1 (C-4″), 99.4 (C-1″), 103.5 (C-8), 115.3 (C-3′,5′), 115.8 (C-6), 118.1 (C-4a), 122.8 (C-1′), 123.4 (C-3), 126.8 (C-5), 130.3 (C-2′,6′), 154.7 (C-2), 155.7 (C-4′), 157.1 (C-8a), 161.0 (C-7), 175.1 (C-6″), 177.8 (C-4); MS (ES^-) m/z 429 (M−H, 100%). Calcd for $C_{21}H_{17}O_7$ 429.0822. Found 429.0829.