Synthesis of Isoflavone Conjugates

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ACADEMIC DISSERTATION

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ABSTRACT

Isoflavones are polyphenols which are found in the plant kingdom, mainly in legumes. The interest towards isoflavones has grown during the years, mainly because of their reported beneficial health effects. Isoflavones exist in plants as their glycosides and are readily hydrolysed in mammals to their free forms, mainly daidzein, genistein and glycitein. These hydrolysed isoflavones are further metabolised to more water soluble compounds, like sulphates, glucuronides and sulphoglucuronides which are then excreted from the body in urine or feces. To understand and reveal the absolute metabolic route of isoflavones, and further study their biological activity, model compounds of these naturally occurring metabolites are needed.

During this study different approaches were studied to obtain isoflavone sulphates, glucuronides and sulphoglucuronides. Three isoflavone disulphates (daidzein-di-O-sulphate, genistein-di-O-sulphate and glycitein-di-O-sulphate) and three isoflavonoid disulphates (dihydrodaidzein-di-O-sulphate, dihydrogenistein-di-O-sulphate and equol-di-O-sulphate) were synthesised in moderate yields by using in situ prepared pyridine sulphur trioxide complex, made from chlorosulphonic acid and pyridine. These disulphated compounds can be used to develop analytical procedures and study the biological activity of disulphated products.

As the use of the HPLC-MS methods in the field of isoflavones has increased its popularity, deuterated isoflavone disulphates were synthesised. A new microwave assisted deuteration method, using CF$_3$COOD, was developed for this purpose. Three polydeuterated isoflavone disulphates (daidzein-$d_6$-di-O-sulphate, genistein-$d_4$-di-O-sulphate and glycitein-$d_6$-di-O-sulphate) were obtained in moderate yields with high isotopic purity.

A synthetic method was developed for daidzein sulphoglucuronide (daidzein-7-O-$\beta$-D-glucuronide-4´-O-sulphate), which is a major metabolite in rat bile. By using protection/deprotection steps, the desired product was finally obtained in moderate yield. The method developed can be used in further studies of synthesis of isoflavonoid mixed conjugates. As a part of this study, the structure of naturally occurring daidzein-4´-O-$\beta$-glucoside was verified. Different glycosidation methods are reviewed and possible factors affecting the stereoselectivity are discussed.

The study of the selective chlorination of isoflavones was a consequence of the observed unexpected chlorination during the synthesis of isoflavone acid chlorides by thionyl chloride. This fascinating phenomenon was investigated further with various isoflavones and as a result a method for producing isoflavone chlorides (8-chlorogenistein, 6,8-dichlorogenistein and 6,8-dichlorobiochanin A) was developed.

Protecting groups played a great role during this study, which led to an intensive study on them. A regioselective protection method was developed by using direct introduction of the protecting group (Benzyl and Benzoyl) to positions 7-O or 4´-O in daidzein, genistein and glycitein with t-BuOK as a base in DMF in moderate yields. The possibility of exploiting the transesterification was also investigated. It was observed that by using K$_2$CO$_3$ as a base in DMF, daidzein, genistein and glycitein could be benzyolated at position 4´-O selectively, in the presence of the more acidic 7 hydroxy group.
Transesterification also proved to be useful in the glycosidation of isoflavones at position 7-\(O\), starting from 7-\(O\)-benzoylated isoflavones.

Different carboxylic acid derivatives were synthesised for use either in the development of radioimmunoassay (7-\(O\)-carboxymethylglycitein and 4'-\(O\)-carboxymethylglycitein) or synthesis of daunorubicin isoflavone derivative for biological testing (7-\(O\)-carboxypropylbiochanin A and 7-\(O\)-carboxypropylgenistein).
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APPREVIATIONS

Ac  Acetyl
Ac₂O Acetic acid anhydride
AcOH Acetic acid
aq. Aqueous
BF₃-Et₂O-Me₂S Boron trifluoride diethyl ether dimethyl sulphide
Bn Benzyl
BnCl Benzyl chloride
n-BuLi n-Butyl lithium
Bz Benzoyle
BzCl Benzoyle chloride
Bu₄NBr Tetrabutylammonium bromide
Bu₃NBr Tetrabutylammonium bromide
Et₃N Triethylamine
Et₂O Diethyl ether
EtOAc Ethyl acetate
EtOH Ethanol
eq. Equivalent
HC(OEt)₃ Triethyl orthoformate
HCO₂Et Ethyl formate
HMBC (¹H-¹³C) Heteronuclear multiple bond correlation (proton-carbon)
HPLC High pressure liquid chromatography
HPLC-MS High pressure liquid chromatography - mass spectrometry
HMOC (¹H-¹³C) Heteronuclear multiple quantum correlation (proton-carbon)
IR Infrared
MDR Multi drug resistance
MeNH₂ Methyl amine
MeOH Methanol
Me₃SiI Trimethylsilyl iodide
MeSO₂Cl Methanesulphonyl chloride
MPLC Medium pressure liquid chromatography
MS Mass spectrometry / Molecular sieve
MW Microwave
N₂H₄-H₂O Hydrazine hydrate
NMP N-Methyl-2-pyrrolidone
NMR Nuclear Magnetic Resonance
Pd/C Palladium on charcoal
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd(OH)$_2$/C</td>
<td>Palladium hydroxide on charcoal (Pearlman’s catalyst)</td>
</tr>
<tr>
<td>Pd(PPh$_3$)$_4$</td>
<td>Tetrakis(triphenylphosphine)palladium(0)</td>
</tr>
<tr>
<td>PhSH</td>
<td>Thiophenol</td>
</tr>
<tr>
<td>pK$_a$</td>
<td>Negative logarithm of the acid ionization constant</td>
</tr>
<tr>
<td>psi</td>
<td>Pound per square inch</td>
</tr>
<tr>
<td>RP</td>
<td>Reverse phase</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>S$_n$1</td>
<td>Unimolecular nucleophilic substitution</td>
</tr>
<tr>
<td>S$_n$2</td>
<td>Bimolecular nucleophilic substitution</td>
</tr>
<tr>
<td>SO$_3$-Me$_3$N</td>
<td>Sulfurtrioxide trimethylamine complex</td>
</tr>
<tr>
<td>SO$_3$-DMF</td>
<td>Sulphur trioxide dimethyl formamide complex</td>
</tr>
<tr>
<td>SO$_3$-pyridine</td>
<td>Sulphur trioxide pyridine complex</td>
</tr>
<tr>
<td>TBAHS</td>
<td>Tetrabutyl ammonium hydrogen sulphate</td>
</tr>
<tr>
<td>TBDMS</td>
<td>t-Butyldimethylsilyl moiety</td>
</tr>
<tr>
<td>TCE</td>
<td>2,2,2-Trichloroethyl</td>
</tr>
<tr>
<td>TEMPO</td>
<td>Tetramethylpiperidine nitroxyl</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Trimethylsilyl trifluoromethanesulphonate</td>
</tr>
<tr>
<td>TR-FIA</td>
<td>Time resolved fluorescent immunoassay</td>
</tr>
</tbody>
</table>
STRUCTURES OF THE MONOSACCHARIDES

Penta-\(O\-acetyl\-\beta\-D\-glucopyranose\ (59)\)

Tetra-\(O\-acetyl\-1\-fluoro\-\alpha\-D\-glucopyanosyl\ (61)\)

Tetra-\(O\-acetyl\-1\-bromo\-\alpha\-D\-glucopyanosyl\ (62)\)

Tri-\(O\-acetyl\-1\-bromo\-\alpha\-L\-quinovopyanosyl\ (64)\)

Tri-\(O\-acetyl\-1\-bromo\-\alpha\-D\-glucopyramuronic acid methyl ester\ (70)\)

Tri-\(O\-benzoyl\-1\-bromo\-\alpha\-D\-arabinofuranosyl\ (73)\)

Tetra-\(O\-acetyl\-\β\-D\-ribofuranose\ (81)\)

Tri-\(O\-acetyl\-1\-fluoro\-\alpha\-L\-rhamnopyanosyl\ (83)\)
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**LIST OF COMPOUNDS AND THEIR IUPAC SYSTEMATIC NAMES IN ORDER OF APPEARANCE**

daizdin (1)  
3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl \(\beta\)-D-glucopyranoside

genistin (2)  
5-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl \(\beta\)-D-glucopyranoside

glycitin (3)  
3-(4-hydroxyphenyl)-6-methoxy-4-oxo-4H-chromen-7-yl \(\beta\)-D-glucopyranoside

daizein (4)  
7-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one

genistein (5)  
5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one

glycitein (6)  
7-hydroxy-3-(4-hydroxyphenyl)-6-methoxy-4H-chromen-4-one

estra-1,3,5(10)-triene-3,17\(\beta\)-diol

daizein-7-\(O\)-glucuronide (8)  
3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl-\(\beta\)-D-glucopyranosiduronic acid

genistein-7-\(O\)-glucuronide (9)  
5-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl-\(\beta\)-D-glucopyranosiduronic acid

glycitein-7-\(O\)-glucuronide (10)  
3-(4-hydroxyphenyl)-6-methoxy-4-oxo-4H-chromen-7-yl-\(\beta\)-D-glucopyranosiduronic acid

daizin-7-\(O\)-sulphate (11)  
3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl hydrogen sulphate

daizein-4´-\(O\)-glucuronide (12)  
4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenyl \(\beta\)-D-glucopyranosiduronic acid

genistein-4´-\(O\)-glucuronide (13)
4-(5,7-dihydroxy-4-oxo-4\textit{H}-chromen-3-yl)phenyl \(\beta\)-D-glucopyranosiduronic acid

daidzein-4\(^{-}\)\textit{O}-sulphate (14)
4-(7-hydroxy-4-oxo-4\textit{H}-chromen-3-yl)phenyl hydrogen sulphate

genistein-4\(^{-}\)\textit{O}-sulphate (15)
4-(5,7-dihydroxy-4-oxo-4\textit{H}-chromen-3-yl)phenyl hydrogen sulphate

daidzein-7\(^{-}\)\textit{O}-glucuronide-4\(^{-}\)\textit{O}-sulphate (16)
4-oxo-3-[4-(sulphooxy)phenyl]-4\textit{H}-chromen-7-yl-\(\beta\)-D-glucopyranosiduronic acid

genistein-7\(^{-}\)\textit{O}-glucuronide-4\(^{-}\)\textit{O}-sulphate (17)
5-hydroxy-4-oxo-3-[4-(sulphooxy)phenyl]-4\textit{H}-chromen-7-yl \(\beta\)-D-glucopyranosiduronic acid

daidzein-7,4\(^{-}\)di-\textit{O}-sulphate (18)
4-[4-oxo-7-(sulphooxy)-4\textit{H}-chromen-3-yl]phenyl hydrogen sulphate

equol (19)
3-(4-hydroxyphenyl)chroman-7-ol

dihydrodaidzein (20)
7-hydroxy-3-(4-hydroxyphenyl)-2,3-dihydro-4\textit{H}-chromen-4-one

dihydrogenistein (21)
5,7-dihydroxy-3-(4-hydroxyphenyl)-2,3-dihydro-4\textit{H}-chromen-4-one

3\(^{-}\)\textit{O}-hydroxydaidzein (22)
3-(3,4-dihydroxyphenyl)-7-hydroxy -4\textit{H}-chromen-4-one

\textit{O}-demethylangolensin (23)
1-(2,4-dihydroxyphenyl)-2-(4-hydroxyphenyl)propan-1-one

dihydrodaidzein-7\(^{-}\)\textit{O}-glucuronide (24)
3-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2\textit{H}-chromen-7-yl \(\beta\)-D-glucopyranosiduronic acid

\textit{O}-desmethylangolensin-7\(^{-}\)\textit{O}-glucuronide (25)
3-hydroxy-4-[2-(4-hydroxyphenyl)propanoyl]phenyl \(\beta\)-D-glucopyranosiduronic acid

genistein-7\(^{-}\)\textit{O}-sulphate (26)
5-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4\textit{H}-chromen-7-yl hydrogen sulphate

genistein-7,4\(^{-}\)di-\textit{O}-sulphate (27)
4-[5-hydroxy-4-oxo-7-(sulphooxy)-4\textit{H}-chromen-3-yl]phenyl hydrogen sulphate

equol-7\(^{-}\)\textit{O}-sulphate (28)
3-(4-hydroxyphenyl)-3,4-dihydro-2\textit{H}-chromen-7-yl hydrogen sulphate

equol-4\(^{-}\)\textit{O}-sulphate (29)
4-(7-hydroxy-3,4-dihydro-2H-chromen-3-yl)phenyl hydrogen sulphate

O-desmethylangolensin-7-O-sulphate (30)
3-hydroxy-4-[2-(4-hydroxyphenyl)propanoyl]phenyl hydrogen sulphate

O-desmethylangolensin-4′-O-sulphate (31)
4-[2-(2,4-dihydroxyphenyl)-1-methyl-2-oxoethyl]phenyl hydrogen sulphate

equol-7, 4′-di-O-sulphate (32)
4-[7-(sulphooxy)-3,4-dihydro-2H-chromen-3-yl]phenyl hydrogen sulphate

O-desmethylangolensin-7,4′-di-O-sulphate (33)
4-[2-[2-hydroxy-4-(sulphooxy)phenyl]-1-methyl-2-oxoethyl]phenyl hydrogen sulphate

equol-7-O-glucuronide (34)
3-(4-hydroxyphenyl)-3,4-dihydro-2H-chromen-7-yl β-D-glucopyranosiduronic acid

equol-4′-O-glucuronide (35)
4-(7-hydroxy-3,4-dihydro-2H-chromen-3-yl)phenyl β-D-glucopyranosiduronic acid

O-desmethylangolensin-4′-O-glucuronide (36)
4-[2-(2,4-dihydroxyphenyl)-1-methyl-2-oxoethyl]phenyl β-D-glucopyranosiduronic acid
daidzein-7,4′-di-O-glucuronide (37)
4-[7-(β-D-glucopyranuronosyloxy)-4-oxo-4H-chromen-3-yl]phenyl β-D-glucopyranosiduronic acid

genistein-7,4′-di-O-glucuronide (38)
4-[7-(β-D-glucopyranuronosyloxy)-5-hydroxy-4-oxo-4H-chromen-3-yl]phenyl β-D-glucopyranosiduronic acid

O-desmethylangolensin-7,4′-di-O-glucuronide (39)
4-[2-[4-(β-D-glucopyranuronosyloxy)-2-hydroxyphenyl]-1-methyl-2-oxoethyl]phenyl β-D-glucopyranosiduronic acid

equol-7-O-glucuronide-4′-O-sulphate (40)
3-[4-(sulphooxy)phenyl]-3,4-dihydro-2H-chromen-7-yl β-D-glucopyranosiduronic acid

O-desmethylangolensin-7-O-glucuronide-4′-O-sulphate (41)
3-hydroxy-4-[2-[4-(sulphooxy)phenyl]propanoyl]phenyl β-D-glucopyranosiduronic acid

2, 4-dihydroxydeoxybenzoin (42)
1-(2,4-dihydroxyphenyl)-2-phenylethanone

phenylacetic acid (43)

resorcinol (44)
2,4-dibenzoyloxy-5-methoxy-acetophenone (45)
1-[2,4-bis(benzyloxy)-5-methoxyphenyl]ethanone

4-benzyloxy-benzaldehyde (46)

2,4,4'-benzyloxy-5-methoxy-chalcone (47)
(2E)-3-[4-(benzyloxy)phenyl]-1-[2,4-bis(benzyloxy)-5-methoxyphenyl]prop-2-en-1-one

2,4,4'-benzyloxy-5-methoxy-acetal-chalcone (48)
(2E)-3-[4-(benzyloxy)phenyl]-1-[2,4-bis(benzyloxy)-5-methoxyphenyl]-2-(dimethoxymethyl)prop-2-en-1-one

phenol (49)
phenol sulphate (50)
phenyl hydrogen sulphate

phenol-4-sulphonate (51)
4-hydroxybenzenesulphonic acid

sulfurtrioxide pyridine complex (52)
oxosulphane dioxide - pyridine (1:1)

pyridine (53)

5,7,4'-trihydroxyflavone (54)
5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one

5,7,4'-tri-O-TCE-sulphate flavone (55)
4-oxo-2-(4-{{[2,2,2-trichloroethoxy)sulphonyl]oxy}phenyl)-4H-chromene-5,7-diyl bis(2,2,2-trichloroethyl) bissulfate

5,7,4'-tri-O-sulphate flavone, ammonium salt (56)
triammonium 4-oxo-2-[4-(sulphonatoxy)phenyl]-4H-chromene-5,7-diyl disulphate

quercetin-3-O-glucoside (57)
5,7-bis(benzyloxy)-2-(2,2-diphenyl-1,3-benzodioxol-5-yl)-4-oxo-4H-chromen-3-yl β-D-glucopyranoside

quercetin-3-O-glucuronide (58)
2-((3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl β-D-glucopyranosiduronic acid

penta-O-acetyl-β-glucopyranose (59)
1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose

phenyl-β-acetyl-glucoside (60)
phenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside
tetra-O-acetyl-1-fluoro-α-D-glucopyranosyl (61)
2,3,4,6-tetra-O-acetyl-1-fluoro-α-D-glucopyranosyl

tetra-O-acetyl-1-bromo-α-D-glucopyranosyl (62)
2,3,4,6-tetra-O-acetyl-1-bromo-α-D-glucopyranosyl

genistein-7-O-tetra-acetylglucoside (63)
5-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

tri-O-acetyl-1-bromo-α-L-quinovopyranosyl (64)
2,3,4-tri-O-acetyl-1-bromo-α-L-quinovopyranosyl

daidzein-7-O-tri-acetyl-β-L-quinovopyranose (65)
3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

daidzein-7-O-tetra-acetylglucoside (66)
3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

daidzein-8,3′,5′-trideutero-7-O-β-glucoside (67)
3-(4-hydroxyphenyl-3′,5′-d2)-4-oxo-4H-chromen-8-7-yl β-D-glucopyranoside

ononin (68)
3-(4-methoxyphenyl)-4-oxo-4H-chromen-7-yl β-D-glucopyranoside

formononetin (69)
7-hydroxy-3-(4-methoxyphenyl)-4H-chromen-4-one

tri-O-acetyl-1-bromo-α-D-glucoyanuronic acid methyl ester (70)
2,3,4-tri-O-acetyl-1-Bromo-α-D-glucoyanuronic acid methyl ester

4′-O-Ac-daidzein (71)
3-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenyl acetate

4′,8-dihydroxyzoflavone-7-O-α-D-arabinofurasine (72)
8-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl α-D-arabinofuranoside

tri-O-benzoyl-1-bromo-α-D-arabinofuranosyl (73)
2,3,5-tri-O-benzoyl-1-bromo-α-D-arabinofuranosyl

4′-O-hexanoyl-daidzein (74)
4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenyl hexanoate

perbenzoylated D-glucopyranosyl-(N-phenyl)trifluoroacetamide (75)
2,3,4,6-tetra-O-benzoyl-1-O-[(1E)-N-benzyl-2,2,2-trifluorothanimidoyl] D-glucopyranose

4′-O-hexanoyl daidzein 7-O-2,3,4,6-tetra-O-benzyol-β-D-glucopyranoside (76)
4-(4-oxo-7-[(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)oxy]-4H-chromen-3-yl)phenyl hexanoate

genistein-4'-O-glucoside (77)
4-(5,7-dihydroxy-4-oxo-4H-chromen-3-yl)phenyl β-D-glucopyranoside

daizein-4'-O-glucoside (78)
4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenyl β-D-glucopyranoside

genistein-4'-O-α-D-ribofuranoside (79)
4-(5,7-dihydroxy-4-oxo-4H-chromen-3-yl)phenyl 2,3,5-tri-O-acetyl-α-D-ribofuranoside

genistein-4'-O-β-D-ribofuranoside (80)
4-(5,7-dihydroxy-4-oxo-4H-chromen-3-yl)phenyl 2,3,5-tri-O-acetyl-β-D-ribofuranoside

1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (81)

daidzein-4'-O-α-L-rhamnopyranoside (82)
4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenyl α-L-rhamnopyranoside

tri-O-acetyl-1-fluoro-α-L-rhamnopyranosyl (83)
2,3,4- tri-O-acetyl-1-fluoro-α-L-rhamnopyranosyl

3',5'-dideuteroformononetin (84)
7-hydroxy-3-(4-methoxyphenyl-3,5-d2)-4H-chromen-4-one

3,5-dideutero-4-methoxybenzaldehyde (85)
4-methoxy-3,5-d2-benzaldehyde

6,8,3',5'-tetradeuteroaidzein (86)
7-hydroxy-3-(4-hydroxyphenyl-3,5-d2)-4H-chromen-4-one-6,8-d2

6,8,3',5'-tetradeuterogenistein (87)
5,7-dihydroxy-3-(4-hydroxyphenyl-3,5-d2)-4H-chromen-4-one-6,8-d2

6,8,2,3',5,6'-hexadeuteroidaidzein (88)
7-hydroxy-3-(4-hydroxyphenyl-2,3,5,6-d4)-4H-chromen-4-one-6,8-d2

7-O-ethoxycarbonylpropyldaidzein (89)
ethyl 4-[[3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl]oxy]butanoate

4'-O-ethoxycarbonylmethylaidzein (90)
ethyl [4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenoxy]acetate

4'-O-ethoxycarbonylmethylgenistein (91)
ethyl [4-(5,7-dihydroxy-4-oxo-4H-chromen-3-yl)phenoxy]acetate

7-O-ethoxycarbonylmethylaidzein (92)
ethyl \{[3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl]oxy\}acetate

7-O-ethoxycarbonylmethylgenistein (93)
ethyl [4-(5,7-dihydroxy-4-oxo-4H-chromen-3-yl)phenoxy]acetate

7-O-carboxymethyl daidzein (94)
\{[3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl]oxy\}acetic acid

7-O-Bn-genistein (95)
7-(benzyl oxy)-5-hydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one

7-O-Bn-daidzein (96)
7-(benzyl oxy)-3-(4-hydroxyphenyl)-4H-chromen-4-one

7-O-Bz-daidzein (97)
3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl benzoate

7-O-Bz-genistein (98)
5-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl benzoate

2-hydroxy-4-methoxy-acetophenone (99)
1-[4-(benzyl oxy)-2-hydroxyphenyl]ethanone

4-methoxymethoxy-benzaldehyde (100)
4-(methoxyethoxy)benzaldehyde

di-O-Ac-daidzein (101)
4-[7-(acetyl oxy)-4-oxo-4H-chromen-3-yl]phenyl acetate

4'-O-Bz-genistein (102)
4-(5,7-dihydroxy-4-oxo-4H-chromen-3-yl)phenyl benzoate

di-O-Bz-genistein (103)
4-[7-(benzoyl oxy)-5-hydroxy-4-oxo-4H-chromen-3-yl]phenyl benzoate

4'-O-TBDMS-genistein (104)
3-(4-\{[tert-butyl(dimethyl)silyl]oxy\}phenyl)-5,7-dihydroxy-4H-chromen-4-one

7,4'-di-O-TBDMS-genistein (105)
7-\{[tert-butyl(dimethyl)silyl]oxy\}-3-(4-\{[tert-butyl(dimethyl)silyl]oxy\}phenyl)-5-hydroxy-4H-chromen-4-one

8-chloro-3',4',5,7-tetrahydroxyisoflavone (106)
8-chloro-3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4H-chromen-4-one

3-chloro-4,6-dimethoxyacetophenone (107)
1-(3-chloro-2-hydroxy-4,6-dimethoxyphenyl)ethanone

6,8-dichlorogenistein (108)
6,8-dichloro-5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one

8,3′-dichlorodaidzein (109)
8-chloro-3-(3-chloro-4-hydroxyphenyl)-7-hydroxy-4H-chromen-4-one

8,3′,5′-trichlorodaidzein (110)
8-chloro-3-(3,5-dichloro-4-hydroxyphenyl)-7-hydroxy-4H-chromen-4-one
daunorubicin (111)
(1S,3S)-3-acetyl-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracene-1-yl 3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranoside

4-methoxyresorcinol (112)
1,3-dihydroxy-4-methoxybenzene

4-hydroxyphenylacetonitrile (113)
(4-hydroxyphenyl)acetonitrile

isovanillin (114)
3-hydroxy-4-methoxybenzaldehyde

2,4,4′-trihydroxy-5-methoxy-deoxybenzoin (115)
1-(2,4-dihydroxy-5-methoxyphenyl)-2-(4-hydroxyphenyl)ethanone

7-O-formylglycitein (116)
3-(4-hydroxyphenyl)-6-methoxy-4-oxo-4H-chromen-7-yl formate

ketiminium chloride intermediate of glycine deoxybenzoin (117)
4-[2-(4-hydroxyphenyl)ethanimidoyl]-6-methoxybenzene-1,3-diol hydrochloride
glycitein-7,4′-di-O-sulphate (118)
4-[6-methoxy-4-oxo-7-(sulphoxy)-4H-chromen-3-yl]phenyl hydrogen sulphate

dihydrodaidzein-7,4′-di-O-sulphate disodium salt (119)
disodium 4-[4-oxo-7-(sulphonatoxy)-3,4-dihydro-2H-chromen-3-yl]phenyl sulphate
dihydrogenistein-7,4′-di-O-sulphate disodium salt (120)
disodium 4-[5-hydroxy-4-oxo-7-(sulphonatoxy)-3,4-dihydro-2H-chromen-3-yl]phenyl sulphate

6,8,2′,3′,5′,6′-hexadeuterogenistein (121)
5,7-dihydroxy-3-(4-hydroxyphenyl-2,3,5,6-d$_4$)-4H-chromen-4-one-6,8-d$_2$

2′,3′,5′,6′-tetradesterogenestein (122)
5,7-dihydroxy-3-(4-hydroxyphenyl-2,3,5,6-d$_4$)-4H-chromen-4-one

5,8,2′,3′,5′,6′-hexadeuteroglycitein (123)
7-hydroxy-3-(4-hydroxyphenyl-2,3,5,6-d$_4$)-6-methoxy-4H-chromen-4-one-5,8-d$_2$
2’,3’,5’,6’-tetradeterogenistein-7,4’-di-\(O\)-sulphate disodium salt (124)
disodium 4-[5-hydroxy-4-oxo-7-(sulphonatoxy)-4\(H\)-chrlomen-3-yl]phenyl 2,3,5,6-\(d_4\) sulphate

6,8,2’,3’,5’,6’-hexadeuterodaizdein-7,4’-di-\(O\)-sulphate disodium salt (125)
disodium 4-[4-oxo-7-(sulphonatoxy)-4\(H\)-chrlomen-3-y1 6,8-\(d_2\)phenyl 2,3,5,6-\(d_4\) sulphate

5,8,2’,3’,5’,6’-hexadeuteroglycitein-7,4’-di-\(O\)-sulphate disodium salt (126)
disodium 4-[6-methoxy-4-oxo-7-(sulphonatoxy)-4\(H\)-chrlomen-3-y1-5,8-\(d_2\)phenyl-2,3,5,6-\(d_4\) sulphate

daizdein-4’-\(O\)-Bn-7-\(O\)-triacetylgucuronic acid methyl ester (127)
3-[4-(benzyloxy)phenyl]-4-oxo-4\(H\)-chrlomen-7-yl methyl 2,3,4-tri-\(O\)-acetyl-\(\beta\)-D-glucopyranosiduronate

daizdein-7-\(O\)-triacetylgucuronic acid methyl ester (128)
3-(4-hydroxyphenyl)-4-oxo-4\(H\)-chrlomen-7-yl methyl 2,3,4-tri-\(O\)-acetyl-\(\beta\)-D-glucopyranosiduronate

daizdein-4’-\(O\)-sulphate-7-\(O\)-triacetylgucuronic acid methyl ester (129)
methyl 4-oxo-3-[4-(sulphooxy)phenyl]-4\(H\)-chrlomen-7-yl 2,3,4-tri-\(O\)-acetyl-\(\beta\)-D-glucopyranosiduronate

4’-\(O\)-Bn-daizdein (130)
3-[4-(benzyloxy)phenyl]-7-hydroxy-4\(H\)-chrlomen-4-one

8-chlorogenistein (131)
8-chloro-5,7-dihydroxy-3-(4-hydroxyphenyl)-4\(H\)-chrlomen-4-one

6-chlorogenistein (132)
6-chloro-5,7-dihydroxy-3-(4-hydroxyphenyl)-4\(H\)-chrlomen-4-one

6,3’-dichlorogenistein (133)
6-chloro-3-(3-chloro-4-hydroxyphenyl)-5,7-dihydroxy-4\(H\)-chrlomen-4-one

biochanin A (134)
5,7-dihydroxy-3-(4-methoxyphenyl)-4\(H\)-chrlomen-4-one

5,7,4’-tri-methoxy-genistein (135)
5,7-dimethoxy-3-(4-methoxyphenyl)-4\(H\)-chrlomen-4-one

6,8-dichlorobiochanin A (136)
6,8-dichloro-5,7-dihydroxy-3-(4-methoxyphenyl)-4\(H\)-chrlomen-4-one

1,1’,1”-(chloro-\(\lambda^4\)-sulphanetriyl)tribenzene (137)
phenolchlorosulphinate (138)
phenyl chloridosulphite
7-O-Bn-glycitein (139)
7-(benzyloxy)-3-(4-hydroxyphenyl)-6-methoxy-4H-chromen-4-one

7-O-Bz-glycitein (140)
3-(4-hydroxyphenyl)-6-methoxy-4-oxo-4H-chromen-7-yl benzoate

7-O-Ac-daidzein (141)
3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl acetate

4’-O-Bn-genistein (142)
3-[4-(benzyloxy)phenyl]-5,7-dihydroxy-4H-chromen-4-one

4’-O-Bn-glycitein (143)
3-[4-(benzyloxy)phenyl]-7-hydroxy-6-methoxy-4H-chromen-4-one

4’-O-Bz-daidzein (144)
4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenyl benzoate

4’-O-Bz-glycitein (145)
4-(7-hydroxy-6-methoxy-4-oxo-4H-chromen-3-yl)phenyl benzoate

7-O-carboxylglycitein (146)
{[3-(4-hydroxyphenyl)-6-methoxy-4-oxo-4H-chromen-7-yl]oxy}acetic acid

4’-O-carboxylglycitein (147)
[4-(7-hydroxy-6-methoxy-4-oxo-4H-chromen-3-yl)phenoxy]acetic acid

daidzein-7-O-Bn-4’-O-tetraacetylglucoside (148)
4-[7-(benzyloxy)-4-oxo-4H-chromen-3-yl]phenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

daidzein-4’-O-tetraacetylglucoside (149)
4-(7-hydroxy-4-oxo-4H-chromen-3-yl)phenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside

daidzein-4’-O-Bz-7-O-triacetylglucuronide methyl ester (150)
3-[4-(benzyloxy)phenyl]-4-oxo-4H-chromen-7-yl methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosiduronate

7-O-(phthalimide-N')butylbiochanin A (151)
2-(4-{{[5-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromen-7-yl]oxy}butyl}-1H-isoindole-1,3(2H)-dione

5-(4-aminobutoxy)-2-[4-(4-methoxyphenyl)-1H-pyrazol-3-yl]benzene-1,3-diol (152)

biochanin A daunorubicin (153)
3-acetyl-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1,2,3,4,6,11-hexahydrotetracen-1-yl 2,3,6-trideoxy-3-{{[5-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromen-7-yl]oxy}butanoyl}amino]hexopyranoside
7-O-carboxypropylbiochanin A (154)
4-{[5-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromen-7-y]oxy}butanoic acid

7-O-carboxypropyl-pivaloylester-biochanin A (155)
2,2-dimethylpropanoic 4-{[5-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromen-7-y]oxy}butanoic anhydride

4′-O-ethoxycarbonylmethylglycitein (156)
ethyl [4-(7-hydroxy-6-methoxy-4-oxo-4H-chromen-3-yl)phenoxy]acetate

7-O-ethoxycarbonylmethylglycitein (157)
ethyl {[3-(4-hydroxyphenyl)-6-methoxy-4-oxo-4H-chromen-7-y]oxy}acetate

7-O-ethoxycarbonylpropylgenistein (158)
ethyl 4-{[5-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-y]oxy}butanoate

7-O-carboxypropylgenistein (159)
4-{[5-hydroxy-3-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-y]oxy}butanoic acid

7-O-ethoxycarbonylpropyl Biochanin A (160)
ethyl 4-{[5-hydroxy-3-(4-methoxyphenyl)-4-oxo-4H-chromen-7-y]oxy}butanoate
1. INTRODUCTION

Isoflavones are polyphenols which are found in the plant kingdom, mainly in legumes. As many as over 870 different isoflavones have been identified in plants.\(^1\) These exist mainly as glycosides, and to be more accurate, as 7-O-β-glycoside conjugates. In human diet, a common source of isoflavones is soy, which is an ingredient used in numerous food products nowadays.\(^2\) The scientific interest towards isoflavones has grown during the past years because of their possible beneficial health effects. This can also be seen in the growing number of the nutritional health products containing isoflavones. Connections with the prevention of cancer, decreased alcohol consumption, prevention of osteoporosis and effect on cardiovascular diseases have been observed.\(^3\) This, however, does not mean that eating products containing isoflavones are the ultimate solution in preventing diseases. In many cases, to obtain the health effects, isoflavones should be eaten in amounts that are impossible to consume, without the risk of toxic side-effects.

**Structural features**

The structure of an isoflavone is composed of a 3-phenylchromen-4-one skeleton, in which the rings are indicated with letters A, C and B, starting from the left (Figure 1). The structure of an isoflavone differs from the structure of a flavone by the position of the phenyl ring, B, which in the case of a flavone is in position 2 (Figure 1). Despite the structural similarities, the chemical behaviour of these compounds differ from each other a great deal, leading to the fact that synthetic methods for flavones cannot be used with isoflavones as such.

![Flavone skeleton](image1)

![Isoflavone skeleton](image2)

**Figure 1.** Flavone and isoflavone skeletons

Isoflavones are a subgroup of the large isoflavonoid family which contains the following groups: isoflavones, isoflavanones, isoflavans, isoflavanols, isoflav-3-enes, α-methyldeoxybenzoins, rotenoids, pterocarpans, coumestans, 2-arylbenzofurans, 3-arylcoumarins and coumaronochromones (Figure 2).\(^4\)
As mentioned earlier, isoflavones exist in plants mainly in their glycoside form. As an example, we may consider a compound such as daidzin (1) (Figure 3). This is one of the most common isoflavone in nature. Other forms of the glycosides are the malonyl-β-glucoside and acetyl-β-glucoside. The β-anomer of the isoflavone glycoside is the main stereochemical form of the compound concerned. This means that the oxygen substituent at carbon C-1′′ (anomeric carbon) is cis to the group at highest numbered chiral carbon atom (C-5′′), as seen in Figure 3. In the case of the minor stereoisomer α-anomer, the bonds are trans to each other. In this thesis the term glycoside will be used to describe the O-glycosides of isoflavones and term glycosidation to describe how they are synthesised.

**Figure 2. Isoflavonoid family**

**Glycoside form**
Despite the fact that isoflavones exist mainly as glycosides in nature, the study in the field of synthesis and research is mainly concentrated on their aglycones, in other words, isoflavones without the monosaccharide moiety. This is mainly because the isoflavone glycosides are readily hydrolysed in mammals to aglycones. Hydrolysis can also take place during the fermentation and germination processes of the plant products. In the literature, both aglycon and aglucon are frequently used terms to describe this form of the structure. In this thesis, the term aglycon is used to describe isoflavones without a monosaccharide moiety. As an example, we may consider the structure of daidzein (4), the aglycon form of daidzin (1) (Figure 4). Other main isoflavone aglycones are genistein (5) and glycitein (6). The common structural feature of these compounds is the phenolic OH groups at positions 7 and 4’. This is also the structural linkage between the female hormone estradiol-17β (7) and 7,4’-dihydroxyisoflavones (Figure 4). The distance between the hydroxy groups in estradiol-17β (7) is nearly the same as in isoflavones (the distance between OH groups at positions 7 and 4’). In theory, this could mean that isoflavones could bind to the same receptors as estradiol-17β (7) creating functions in the human body which are yet unknown.
As a result of the hydrolysis, the formed aglycones are further metabolised to phenolic conjugates in the human body. These include sulphates, glucuronides and sulphoglucuronides which are highly water soluble conjugates (Figure 5). The aglycones can also be de-methylated, reduced and oxidised (Figure 6). Neither the biological activity nor the exact metabolic route of the metabolised compounds are fully understood. It remains to be seen if the further metabolised compounds have a stronger biological activity than the aglycon forms.

The following chapters will concentrate on the background of isoflavones and their metabolites and on the synthesis of isoflavone O-conjugates which include sulphates and glucosides. The methods for regioselective alkylation and protection will be discussed, including the cleavage of the protecting groups. The chapter 3, Results and Discussion, will present the completed work during this research. Methods for sulphation and glucuronation of isoflavones, regioselective protection and selective deprotection, O-alkylation and chlorination of isoflavones will be presented.

1.1 SOURCES OF ISOFLAVONES IN HUMAN DIET

In the previous chapter, it was stated that isoflavones exist in plants mainly as their 7-O-glycosides, such as daidzin (1),\textsuperscript{10} genistin (2)\textsuperscript{11} and glycitin (3) (Figure 3).\textsuperscript{12} Isoflavone glycosides can be found in various natural sources including seeds, beans, fruits, hops and cherries.\textsuperscript{3} The common source of isoflavones is soy, containing significant amounts of isoflavones. As an example, soy flour contains up to 2 mg/g of isoflavones.\textsuperscript{13} By comparison, other sources contain only traces of these beneficial compounds, (µg/g). The average daily consumption of soy protein among Chinese women in Asia was estimated to be 36-45 g.\textsuperscript{14} This would mean a 500 g average consumption of soy products (e.g. tofu, miso and soy milk) per day and a 40 mg daily intake of isoflavones.\textsuperscript{13,14} In Western countries the daily consumption of soy is low, since the usual sources of protein are animal based products.
1.2 METABOLISM OF ISOFLAVONES

Numerous studies have been conducted concerning the metabolism of isoflavones in mammals. These studies have mostly been based on the results of various HPLC-MS data of physiological matrices.\textsuperscript{15,16,17,18,19,20,21,22,23,24,25,26,27,28,29} There are only few GC-MS studies owing to the required additional derivatisation steps.\textsuperscript{30,31} It is noteworthy that the sensitivity of GC-MS is, however, better than HPLC-MS. The basic idea of the HPLC-MS studies is to identify isoflavones and their metabolites (qualitative analysis) and compare their concentrations in the samples, for example in urine, plasma, and serum (quantitative analysis). In the HPLC based studies, the samples are usually hydrolysed enzymatically, which results in misleading data concerning the identification of sulphates and glucuronides and their concentrations in the sample. These studies often suggest that the amount of aglycones in physiological samples from humans is predominating. As we will see later, this is not the absolute truth. This matter should be taken into account, when testing the biological activities of isoflavones. The reliability of the analytical procedures suffers from the lack of suitable model compounds. These are crucial in testing the behaviour and to find out the stability of the analytes during the analysis procedure.

At first step, isoflavone glucosides are hydrolysed by intestinal flora to their aglycones, such as daidzin (1) to daidzein (4). In 2002 Setchell \textit{et al.} proved that isoflavone glucosides do not exist in plasma, supporting the fact that they are hydrolysed before entering the blood stream.\textsuperscript{32} Aglycones are mainly metabolised to sulphate, glucuronide and sulphoglucuronide conjugates, making isoflavones more water soluble, thus improving their excretion (Figure 5).
**Figure 5.** Isolated mammalian hydrophilic metabolites of isoflavones

On the other hand, these hydrophilic metabolites and aglycones can be further metabolised to equol (19), dihydrodaidzein (20), dihydrogenistein (21), 3'-hydroxydaidzein (22) and O-desmethylangolensin (23), although in lower amounts (Figure 6). These can then be remetabolised to sulphates, glucuronides and sulphoglucuronides (Figure 7).
Figure 6. Isolated reduced and oxidised mammalian metabolites of isoflavones

Figure 7. Isolated glucuronides of reduced isoflavones

King presented the following figure describing the possible metabolic routes of isoflavones in mammals (Figure 8). In the represented figure, solid arrows show the movement of isoflavones and the dashed ones their bacterial conversion.
Figure 8. Circulation of isoflavones in mammals

There have also been a few studies in the field of isolation and identification of isoflavone metabolites in rats and humans. In 2002, a study was published by Ohsawa et al. on the mammalian metabolism of *Radix puerariae* isoflavonoids. *Radix puerariae* is the root of *Pueraria lobata* Ohwi, a perennial plant found in Asia. The root, from which isoflavonoids can be found, is used as a herbal medicine. Ohsawa et al. isolated various conjugates of isoflavones, mainly conjugates of daidzein (4) and genistein (5), from urine,
bile and feces (Table 1). They analysed the isolated compounds by using IR, MS and NMR techniques. One interesting observation was that daidzein-7-O-glucuronide-4′-O-sulphate (16) was detected in bile but not in urine. They thought that the reason for this behaviour may be explained by the structure of daidzein-7-O-glucuronide-4′-O-sulphate (16). As being a relatively large compound and with high polarity, it might be excreted rather via bile than urine. This is important to take a notice of in the development of analytical studies which try to estimate the total amount of isoflavones and their metabolites by analysing urine. As mentioned earlier, the hydrolysis of HPLC samples can give misleading results as well as if the study is based only on the analysis of urine. The found isoflavone metabolites and their sources (e.g. urine, bile, plasma, feces) are collected in Table 1, where it can be seen that the analytical study has concentrated mainly on rats. The few results of human metabolism found in urine, are in contradicting. It seems that there is no clear agreement for the sulphation and glucuronidation sites in isoflavones. Further studies are needed by analysing human urine with controlled feeding. The use of internal standards should also be considered.
Table 1. Detection of isoflavones and their metabolites from biological samples

<table>
<thead>
<tr>
<th>Source/isoflavone/</th>
<th>Ref.</th>
<th>Method</th>
<th>Aglycones</th>
<th>Sulphates</th>
<th>Glucuronides</th>
<th>Sulpho-glucuronides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat urine/puerarin</td>
<td>34</td>
<td>isolation</td>
<td>4</td>
<td>14, 18</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rat urine/daidzin/after hydrolysis</td>
<td>35</td>
<td>isolation</td>
<td>4, 19 20, 22</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Rat urine/daidzin, daidzein</td>
<td>38</td>
<td>isolation</td>
<td>4</td>
<td>14, 18</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rat urine/daidzin, daidzein</td>
<td>40</td>
<td>HPLC</td>
<td>4, 19, 20, 22</td>
<td>14, 18</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rat bile/ daidzin, daidzein</td>
<td>40</td>
<td>HPLC</td>
<td>4</td>
<td>14, 18</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Rat plasma/ daidzin, daidzein</td>
<td>40</td>
<td>HPLC</td>
<td>4</td>
<td>14, 18</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rat feces/ daidzin, daidzein</td>
<td>40</td>
<td>HPLC</td>
<td>4</td>
<td>14, 18</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rat urine/ daidzin, daidzein</td>
<td>38</td>
<td>HPLC</td>
<td>4</td>
<td>14, 18</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Rat bile/ daidzin, daidzein</td>
<td>38</td>
<td>HPLC</td>
<td>4</td>
<td>14, 18</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Human urine/soy supplement</td>
<td>39</td>
<td>isolation</td>
<td>4, 5, 6, 20, 23</td>
<td>8, 9, 10, 12, 13, 24, 25</td>
<td>16, 17</td>
<td></td>
</tr>
<tr>
<td>Rat urine/genistein</td>
<td>36</td>
<td>isolation</td>
<td>5</td>
<td>15</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Rat urine/genistein</td>
<td>36</td>
<td>HPLC</td>
<td>5</td>
<td>15</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Rat bile/genistein</td>
<td>36</td>
<td>HPLC</td>
<td>5</td>
<td>15</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Rat urine/genistein/after hydrolysis</td>
<td>37</td>
<td>isolation</td>
<td>19, 21</td>
<td>8, 9, 12, 13, 37, 38</td>
<td>16, 17</td>
<td></td>
</tr>
<tr>
<td>Human urine/soy supplement</td>
<td>31</td>
<td>GC/MS</td>
<td>4, 5, 19, 23</td>
<td>11/14, 26/15, 28/29, 30/31, 32, 33</td>
<td>8/12, 9/13, 34/35, 25/36, 37, 38, 39</td>
<td>16, 17, 40, 41</td>
</tr>
<tr>
<td>Human urine/soy supplement</td>
<td>19</td>
<td>LC/MS</td>
<td>4, 5</td>
<td>11, 26, 14, 15</td>
<td>8, 9, 12, 13, 37, 38</td>
<td>16, 17</td>
</tr>
</tbody>
</table>

Bold numbers indicate isolated and detected compounds (See Figure 5, 6 and 7 for details)
1.3 BIOLOGICAL ACTIVITY OF METABOLITES

There exist various publications on the biological activity of isoflavones and their possible health effects.\textsuperscript{41,42,43,44,45,46,47,48,49} Biological activity studies have been mainly concentrated on daidzein (4), genistein (5) and also on their glucosides because these are readily hydrolysed in mammals (rats and humans) to aglycones. It has to be taken into account, that the actual active compound might be one of the metabolites (e.g. glucuronides, sulphates, reduced or oxidised) rather than aglycones. As an example we may consider daidzein-7,4′-di-O-sulphate (18), the main bile metabolite in rats. Daidzein-7,4′-di-O-sulphate (18) is a potent inhibitor of sterol sulphatase.\textsuperscript{50} Sterol sulphatase is the enzyme that releases estrogen from its inactive sulpho form and by doing this makes the estrogen available in the human body. Interaction with the estrogen receptor has also been studied with glycitein (6)\textsuperscript{51} and with daidzein or genistein sulpho and sulphoglucuronides.\textsuperscript{46}

1.4 SYNTHESIS OF ISOFLAVONES AND THEIR CONJUGATES

The need for the development of synthetic methods for the title compounds is obvious. Especially authentic samples for the confirmation of the isolated plant conjugates and their human metabolites are necessary for the testing of the possible biological activity of the identified compounds. This chapter concentrates on the synthesis of the most common isoflavones; daidzein (4), genistein (5) and glycitein (6), and on the synthesis of their various O-conjugates, which are divided into the separated subchapters (Chapters 1.4.2-1.4.5). First, the synthesis of starting isoflavone aglycones will be introduced. Next, methods available for sulphation, glycosidation, deuteration and regioselective O-substitution of isoflavones as well as chlorination, will be discussed.

1.4.1 SYNTHESIS OF ISOFLAVONE AGLYCONES

The first synthesis of isoflavone aglycones was published in 1925 by Baker \textit{et al.}\textsuperscript{52} They synthesised various 2-substituted isoflavones starting from 2-hydroxydeoxybenzoins. This was the beginning of the synthetic study of isoflavones.\textsuperscript{53,54} The two most popular synthetic pathways to isoflavones are the deoxybenzoin (2-hydroxyphenyl benzyl ketone)\textsuperscript{55,56,57,58,59,60,61,62} and the chalcone\textsuperscript{53,64,65,66,67,68,69,70,71,72} routes. These routes are presented in Schemes 5 and 7, respectively. Other not that commonly used routes include the conversion of a flavanone (Scheme 1),\textsuperscript{73,74,75} epoxidation of a chalcone (Scheme 2),\textsuperscript{76,77} condensation of an enamine with salicylaldehydes (Scheme 3)\textsuperscript{78} and the well known Suzuki cross-coupling reaction (Scheme 4),\textsuperscript{79} all yielding isoflavones.
Scheme 1. Conversion of flavanone to isoflavone

Reagents and conditions: i) Tl(NO$_3$)$_3$, MeOH, CHCl$_3$

Scheme 2. Isoflavone from epoxidated chalcone

Reagents and conditions: i) BF$_3$·Et$_2$O, benzene, +10 °C ii) HCl, AcOH

Scheme 3. Isoflavone by condensation of enamine

Reagents and conditions: i) Benzene, reflux ii) CrO$_3$, pyridine, RT
Reagents and conditions: Pd(PPh₃)₄, Na₂CO₃, benzene, reflux

Scheme 4. Isoflavone by Suzuki cross-coupling

Deoxybenzoin route

The deoxybenzoin intermediate is first formed by standard electrophilic substitution of phenol with benzyl cyanide (Houben-Hoesch reaction) or phenylacetic acid (Friedel-Crafts acylation), followed by cyclisation (Scheme 5).

Reagents and conditions: Houben-Hoesch: (R⁵=CN) HCl, ZnCl₂, Et₂O, RT i) H⁺/H₂O. Friedel-Crafts: (R³=COOH) BF₃·Et₂O. ii) BF₃·Et₂O, MeSO₂Cl, DMF

Scheme 5. Synthesis of deoxybenzoins by the Houben-Hoesch reaction or the Friedel-Crafts acylation

Houben-Hoesch

The synthetic approach using the standard Houben-Hoesch reaction employes ZnCl₂ as a Lewis acid, which has been substituted by BF₃·Et₂O complex in one case. The BF₃·Et₂O complex acts as a solvent and a catalyst 'all in one', which can be considered to be an advantage over the use of traditional ZnCl₂ where a dry solvent (ether) is needed.
**Friedel-Crafts acylation**

Panasenko et al. presented the following reaction scheme for Friedel-Crafts acylation of 2,4-dihydroxydeoxybenzoin (42) starting from phenylacetic acid (43) and resorcinol (44). It should be noted that the reaction involves a release of water and hydrogen fluoride. The latter can lead to cleavage of acid sensitive groups.

![Scheme 6. Friedel-Crafts acylation of resorcinol with phenylacetic acid](image)

The last step in the deoxybenzoin route is cyclisation, which can be carried out in many ways. The additional carbon atom needed for the ring formation can be introduced, for example, by the treatment with one of the following combinations of reagents BF$_3$-Et$_2$O/DMF/MeSO$_2$Cl, BF$_3$-Et$_2$O/DMF/PCl$_5$, Zn(CN)$_2$/HCl, HCO$_2$Et/Na, HC(OEt)/pyridine and dimethoxydimethylaminomethane. One option for the cyclisation reaction is to use ethoxallyl chloride (ClCOCO$_2$Et) which gives the isoflavone after the decarboxylation step. In some cases the substitution and ring-closing steps can be combined in a one-pot reaction. The effectiveness of the one-pot reaction depends heavily on the structures of the starting materials. In some cases, the ring closing has been carried out under microwave irradiation without any notable improvements to the yields. The beauty of the deoxybenzoin route is the straightforwardness of the reaction as there is no need for the protection of phenolic OH-groups.

In the majority of the publications, daidzein (4) has been prepared employing the Friedel-Crafts acylation. Among them are three publications on glycitein (6) one using the deoxybenzoin route and two others the chalcone approach. In the case of genistein (5) the situation is more complex. There are reports in which genistein (5) has been produced by one-pot Friedel-Crafts acylation (BF$_3$-Et$_2$O), whereas some studies have applied the Houben-Hoesch approach. The yields are usually high when the deoxybenzoin route is used and as mentioned before, the procedure is rather
straightforward. Although the Houben-Hoesch reaction is often criticised of being laborious, our experiences do not confirm that argument.

**Chalcone route**

The chalcone intermediate is obtained by the condensation of protected acetophenones and benzaldehydes under basic conditions (Scheme 7). The formed chalcone is then oxidatively rearranged usually with thallium (III) salts.

In the synthesis of glycitein (6), acetophenone (45) and benzaldehyde (46) give chalcone (47) (Scheme 7). This is further oxidatively rearranged with thallium (III) salts to give the acetal (48) which is cyclised in acidic conditions to give glycitein (6).

![Scheme 7. Synthesis of glycitein (6) by chalcone route](image)

**Reagents and conditions:** i) 50% aq. KOH, EtOH, RT ii) trimethyl orthoformate, Tl(NO₃)₃, MeOH, RT iii) Pd/C, H₂, MeOH/acetone (1:1), RT iv) conc. HCl, MeOH, reflux

1.4.2 SULPHATION OF ISOFLAVONES

There are only a few examples of the sulphation of isoflavones in the literature. However, several publications concerning the sulphation can be found on flavones and, not surprisingly, numerous on phenols. There are multiple ways to sulphate phenols and these methods can be employed as tools when designing the sulphation reactions for isoflavones and their conjugates. Most of the methods are based on the complexes of sulphur trioxide (SO₃), either with pyridine (Scheme 9), DMF, TCE (Scheme 10), or DCC (Scheme 11). SO₃ by itself is an aggressive electrophilic reagent that rapidly reacts with any organic molecule containing an electron donating group without any selectivity or
In the case of phenol (49), SO₃ can give either sulphate esters (50) or sulphonates (51) (Scheme 8).

Scheme 8. Reaction between phenol and SO₃

The reactivity towards organic compounds can be controlled by coupling SO₃ with a Lewis base, usually with pyridine or DMF. The reactivity and stability of the SO₃ complex, as for sulphation, depend on the strength of the base used. Weaker the base, greater the partial positive charge on sulphur atom on the SO₃ complex. For example, DMF is a weaker base than pyridine, leading to the fact that SO₃ DMF is a stronger sulphating agent than the corresponding pyridine complex, but still mild enough to produce only sulphate esters. One common procedure for making a pyridine sulphur trioxide complex (52) is the reaction between pyridine (53) and chlorosulphonic acid (ClSO₃H) either with isolation of the formed complex (Scheme 9) or in situ.

Scheme 9. Preparation of pyridine sulphur trioxide complex

Reagents and conditions: i) pyridine, ClSO₃H, Et₂O, 0 °C

TCE complex

There are also some examples of the use of 2,2,2-trichloroethyl (TCE) ester of chlorosulphuric acid as a sulphation agent for flavones and estrones (Scheme 10). Here, TCE acts as a protecting group for the sulphate ester and can be cleanly cleaved by hydrogenolysis with ammonium formate (HCOONH₄) and palladium on charcoal (Pd/C) without affecting the sulphate moiety itself. The stability of the aryl TCE sulphates in the highly acidic conditions is remarkable. It also creates possibilities in the purification step by changing the polarity of the compound. This sulphation route could especially be useful in the situation were further reactions are carried out after the sulphation step. In theory, this method could also be used in the regioselective sulphation of isoflavones.
Reagents and conditions: i) TCEOSO₂Cl, TEA, DMAP, THF, RT ii) 10% Pd/C, HCOONH₄, THF-MeOH, RT

Scheme 10. Sulphation of flavone by TCE-complex

**DCC**

In the flavone field, the sulphations have been mainly performed under dicyclohexylcarbodiimide acid (DCC) activated reaction conditions, or by using the sulphur trioxide trimethylamine complex (SO₃-Me₃N), resulting in mixtures of sulphates. In the reaction where sulphuric acid is used, the role of DCC is to work as an activator. Hoiberg *et al.* presented a possible mechanism for the DCC activated reaction with sulphuric acid and phenol in 1969 (Scheme 11). They noticed that the formed O-sulphonylisourea is stable only for 30 seconds after the formation. Barron *et al.* were not able to obtain sulphated flavonoids using the DCC sulphuric acid method. However, they found that changing sulphuric acid to tetrabutylammonium hydrogen sulphate (TBAHS) afforded sulphated flavonoids in moderate yields. The reason for this behaviour might be explained by the salt formation between the O-sulphonylisourea and tetrabutylammonium cation which also stabilises the formed sulphate ester.
Recently Jones et al. reported a synthesis for a mixture of quercetin sulphates using SO$_3$-DMF complex as sulphation reagent. The yields for compounds separated by HPLC ranged from 0.8% to 16.3%.

As for the isoflavones, in 1996 Peterson et al. reported the synthesis of genistein-4′-O-sulphate (15) using sulphuric acid in the presence of DCC in DMF. In 1997 Wong et al. described a synthesis for a mixture of daidzein-4′-O-sulphate (14) and daidzein-di-O-sulphate (18) using the SO$_3$-DMF complex. Both of the above presented publications were published without any data on yields, making it difficult to judge the efficiency of the suggested methods. Fairley et al. synthesised daidzein-di-O-sulphate (18) and mono sulphates (11, 14) using protecting group chemistry (e.g. TBDMS) and SO$_3$-pyridine complex. The approach with TBDMS for isoflavones is quite a different method as the phenolic OH groups are directly and regioselectively protected with TBDMS, after which the sulphation reaction is carried out.
1.4.3 GLYCOSIDATION OF ISOFLAVONES

The literature presented in this chapter mainly focuses on the synthesis of isoflavone 7 and 4’-O-glycosides. This is due to the fact that the monosaccharide moieties are generally located at the position 7-O and 4’-O. These glycoside derivatives can be found in nature, as mentioned earlier in the general introduction part, but these derivatives have also been synthetically prepared. The methods described for the synthesis of glucosides can be used in the synthesis of isoflavone glucuronides. There are only a few publications describing the synthesis towards isoflavone glucuronic acids.¹⁰⁸,¹⁰⁹ One possibility for the synthesis of isoflavone glucuronides is a method described by Bouktaib et al. in 2002 (Scheme 12).¹¹⁰ They were able to oxidise flavonol glucoside (57) into flavonol glucuronide (58) by using protecting groups (e.g. benzyl group) on phenols and a catalytic amount of tetramethylpiperidine nitroxy (TEMPO), as a free radical generator, in the presence of sodium hypochlorite (NaOCl) as an oxidant.

\[ \text{Reagents and conditions: i) NaOCl, TEMPO ii) Pd/C, H}_2 \]

**Scheme 12. Oxidation of flavonol glucoside to flavonol glucuronide**

To understand the stereoselectivity of the glycosidation reactions, different types of mechanisms are introduced. There are two kinds of glycosidation reactions for phenols, those which give only one stereoisomer and those which give mixtures of anomers. The stereospecific reactions are mainly accepted to be $S_N2$ type reactions, while those giving mixtures of anomers are based on the formation of a carbocation ($S_N1$). As a simple example we may consider the reaction between glucose and methanol in a presence of an acid catalyst (Scheme 13).¹¹¹ The reaction is initiated by the cleavage of water which creates a resonance stabilised carbocation which further reacts with nucleophile. The attack of the nucleophile can happen from both sides of the anomic carbon, leading to a mixture of $\alpha$ and $\beta$ anomers, $\beta$ anomer being the major product.
Scheme 13. Glycosidation of glucose with methanol in the presence of acid catalyst

The situation is different when the OH groups are acetylated or benzoylated in the monosaccharide unit. The acetyl or benzoyl group at the C-2 position creates the so-called neighbouring group effect which means that the carbonyl group forms a five-membered ring with the anomeric carbon (Scheme 14). The nucleophilic attack is forced to take place from the β face, leading to the β anomer. The stereochemistry of the starting sugar does not affect the formation.
Scheme 14. Neighbouring group effect in glycosidation reaction leading to β-anomer

The situation is not as simple in the case of phenols, which are much weaker nucleophiles than alcohols. First we will start by a brief introduction to the methods that have been used in the synthesis of phenolic glycosides. The main glycosidation method for phenols is the reaction of phenol with glycosyl fluoride or bromide under various conditions. These include the use of a phase transfer catalyst, \[^{113,114,115,116,117,118}\] Koenigs-Knorr, \[^{119,120,121,122,123,124,125,126}\] silver triflate, \[^{127,128}\] BF\(_3\)-Et\(_2\)O \[^{129,130}\] and Cp\(_2\)ZrCl\(_2\)-AgBF\(_4\). \[^{131}\] Yamaguchi et al. noticed that in the case of BF\(_3\)-Et\(_2\)O, the stereochemistry can be controlled by using various amino bases, and suggested a mechanism for this stereochemical control (Scheme 15). \[^{129}\] The β-anomer is favoured when an amine base is present. This is caused by the removal of the proton from the equatorial position in the intermediate before the transition to axial form, which gives the α-anomer.
Scheme 15. Stereoselectivity of glycosidation reaction in the presence of base

Lee et al. noticed in their glycosidation experiments with penta-O-acetyl-β-glucopyranose (59), that 50% of the formed phenyl-β-acetyl-glucoside (60) was isomerised in the presence of BF₃-Et₂O to its α anomer during 10 hours at room temperature.¹³² The use of triethylamine exhibited the isomerisation. On the other hand, Jensen et al. reported only minor changes in the α/β ratio during four hours, in the presence of BF₃-Et₂O.¹²⁷ However, they did not test the effect of the base in their studies and it should be noted that they used tetra-O-acetyl-α-D-glucopyranosyl fluoride (61) as monosaccharide. The true nature of the BF₃-Et₂O mediated reaction mechanism is still unclear, as is also the influence of the glycoside halide stereochemistry.

Yu et al. used a mixture of α/β acetyl and benzoyl protected glycosyl trifluoro(phenyl)acetamides in the presence of trimethylsilyl trifluoromethanesulphonate (TMSOTf) to obtain β glycosides in moderate yields.¹³³,¹³⁴ Clerici et al. reported the case of penta-O-acetyl-β-glucopyranose (59) where the acetyl group at anomeric position acts as a leaving group. This reaction also follows the mechanism presented in Scheme 14, leading to β anomers.¹³⁵ In this reaction, tin tetrachloride (SnCl₄) forms an adduct with AcO⁻, derived from penta-O-acetyl-β-glucopyranose (59). Clerici et al. noted that the use
of the α anomer glycoside as a starting material led to lower yields compared to the β anomer.\textsuperscript{135}

With two-phase or Koenigs-Knorr reaction conditions, the glycosidation products are usually obtained as one stereoisomer although the use of Koenigs-Knorr conditions does not exclude the possibility of carbocation formation.\textsuperscript{119} The possible side reactions in a two-phase reaction are the β-elimination and hydrolysis of halide.\textsuperscript{136}

In the synthesis of phenolic sugars, the key factor is the stereochemistry. Reaction conditions, the stereochemical purity of the glycosyl donor, as well as reactivity with phenol have to be considered in order to achieve high yields with selective α:β ratio. It also has to be understood that in many cases the α:β ratio can depend on factors such as solvent, temperature, leaving group and substitution stage of phenol. Although the syntheses described above might work well for phenols it is not certain that they work for isoflavones.

In conclusion, it can be said that the two-phase and Koenigs-Knorr methods produce stereoechemically pure compounds. The use of methods where a carbocation is formed, mainly BF\textsubscript{3}-based yields are usually higher for glycosidation but the stereoselectivity depends heavily on several factors. These are the nucleophilicity of the phenol, solvent used, presence of base and reaction temperature. When considering the synthesis of glycosides it has to be decided which factor is of major importance, the high yield or the stereoselectivity of the reaction.

### 1.4.3.1 SYNTHESIS OF ISOFLAVONE-7-O-GLYCOSIDES

This chapter describes the methods used in the synthesis of isoflavone-7-O-glycosides by regioselective glycosidation or by using protecting groups. The first regioselective glycosidation for isoflavones was introduced by Zemplén et al. in 1943.\textsuperscript{137} They synthesised genistin (2), by reacting genistein (5) with tetra-acetyl-O-α-D-glucopyranosyl bromide (62) in a 9% KOH solution in acetone. The reaction gave genistin (2) in a 17% yield after the hydrolysis of the acetyl groups on glucoside moiety. The intermediate, genistein-7-O-tetra-acetylglucoside (63), was not isolated. Several similar types of reactions, without the isolation of the acetyl protected intermediate, have been reported.\textsuperscript{138} Selective glycosidation at position 7-O is possible because of the difference in the acidity between the hydroxy groups 7 and 4′ in polyhydroxyisoflavones (Figure 4). Nishiyama et al. used a 2M KOH solution and tri-O-acetyl-α-L-quinovopyranosyl bromide (64) in acetone to synthesise daidzein-7-O-tri-acetyl-β-L-quinovopyranoside (65) in a 32% yield.\textsuperscript{66} It should be noted that they were able to isolate the acetyl protected intermediate. In 1998 Lewis et al. reported a method for synthesising several isoflavone-7-O-β-glucosides in yields varying from 40% to 42%.\textsuperscript{139} These included the synthesis of daidzin (1) and genistin (2) using a two-phase system with tetrabutylammonium bromide (Bu\textsubscript{4}NBr) as a phase transfer catalyst. Furthermore, they did not isolate the intermediate products, isoflavone-7-O-tetra-acetyl glucosides (63, 66). From that, it can be assumed that this method is not suitable for reactions where the acetyl protecting groups are needed on the glucoside unit for further reactions, as an example, sulphation at the 4′-O-position. In the same year Lewis et al. published another method for the glycosidation of
isoflavones at position 7-O without isolating the acetylated intermediate. Now they used solid-liquid conditions, to produce daidzein-8,3',5'-trideutero-7-O-β-glucoside (67) in a 40% yield. In 2001 Wang et al. reported a synthesis for ononin (68) starting from formononetin (69). They performed the glycosidation step using potassium carbonate (K$_2$CO$_3$) as a base in a solvent mixture of DMF/acetone with dodecyltrimethylammonium bromide (DTMAB) as a phase transfer catalyst. The yield for acetylglicoside isoflavone was 85%. Glycitin (3) was synthesised regioselectively in 1996 by Nógrádi et al. applying the Koenigs-Knorr method, where phenol is reacted with an α-glucoside halide using silver salts, usually silver carbonate (Ag$_2$CO$_3$), or pyridine as an acid acceptor to give β-glycoside. The yield for the glycosidation step was 17%. Needs et al. investigated the same method to synthesise daidzein-7-O-β-D-glucuronide (8) by using tri-O-acetyl-α-D-glucopyranuronic acid methyl ester bromide (70). Moreover, they noticed that without the protection of the 4'-OH, the desired reaction did not take place. Instead of the target compound, only 4'-O-Ac-daidzein (71) with unidentified groups at position 7 were detected. When acetyl protection was used at position 4'-O, the yield of the glucuronidation step was 30%. Koenigs-Knorr reaction has been used in several other syntheses of isoflavone glucosides with protecting groups.

In 1999, Shiozaki published a method for synthesising 4',8-dihydroxyisoflavone-7-O-α-D-arabinofurasine (72) using allyl protection. The glycosidation was performed using n-butyl lithium (n-BuLi) as a base in THF to generate the isoflavone lithium salt which was further reacted with tri-O-benzoyl-1-bromo-α-D-arabinofuranosyl (73) to yield 26% of the corresponding protected glycoside. It should be noted that the stereochemistry remained the same during the reaction, meaning that the reaction mechanism is not S$_2$2. The reason for this stereochemistry is the neighbouring group effect which leads to the attack from the α face, since the Bz and Br are trans to each other in starting material (see previous chapter for details). One option for the glycosidation of isoflavones is a trifluoroacetamide approach, where the protected isoflavone is reacted with glycosyl (N-phenyl)trifluoroacetamide (mixture of α and β anomers) in the presence of BF$_3$-Et$_2$O to give the corresponding isoflavone glycoside up to 90% yields (Scheme 16).
Reagents and conditions: BF$_3$-Et$_2$O, 4Å MS, CH$_2$Cl$_2$, RT

Scheme 16. Glycosidation of isoflavone by trifluoroacetamide monosaccharide

As a conclusion, it can be said that there is no selective method available for the synthesis of isoflavone-7-O-β-glycosides, in which the hydroxy groups at the monosaccharide moiety are protected with acetyl groups. It seems that the only practical methods are those using a protecting group at position 4'-O on the isoflavone.

1.4.3.2 SYNTHESIS OF ISOFLAVONE-4'-O-GLYCOSIDES

Isoflavone-4'-O-glycosides are rare in the nature, and so are the synthetic publications. In fact, there are only a few publications describing the synthesis of them. In 1954, Bognár et al. introduced a method for synthesising genistein-4'-O-β-glucoside (77), also known as sophoricoside (77), using p-nitro-benzyl protection at position 7-O.\(^{145,146}\) The yield for the glycosidation step, performed with a 2.5% KOH solution, was only 6%. 44 years later Lewis et al. reported a regioselective method for the 4'-O-β-glycosidation of isoflavones (77, 78) using 18-crown-6 as a phase transfer catalyst and t-BuOK as a base in acetonitrile.\(^{147}\) However, the reported NMR data is rather confusing. The signal at 9.60 ppm in the spectrum of sophoricoside (77) indicates that the 4'-OH is unconjugated. It seemed that in case of genistein (5) they in fact synthesised genistin (2). This will be discussed later in Chapter 3.6.3. In 2001, Boryski et al. published a method for synthesising genistein-4'-O-ribofuranosides (79, 80) (Scheme 17).\(^{148}\) They used SnCl$_4$ and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose (81) for regioselective synthesis. The reaction gave a mixture of α and β products in a ratio of 1:2. The yield of β-anomer after column chromatographic purification was 54%. These results are in agreement with the previously
published method for phenols, which used penta-\(O\)-acetyl-\(\beta\)-glucopyranose (59) as starting material (Chapter 1.4.3).\(^{135}\) Boryski \textit{et al.} did not investigate the effect of the stereochemistry of the starting monosaccharide.\(^{148}\) Instead, they noticed that the use of a acetonitrile/\(\text{CH}_2\text{Cl}_2\) solvent mixture in the reaction improved the yields notably.

![Diagram](image)

\textit{Reagents and conditions:} i) glucoside, \(\text{SnCl}_4\), \(\text{CH}_2\text{Cl}_2\), RT ii) genistein, \(\text{CH}_3\text{CN}\), RT

**Scheme 17.** Synthesis of genistein-4-\(O\)-glycosides

Nishiyama \textit{et al.} used TMS derivatisation for isoflavone to enhance the nucleophility in the synthesis of daidzein-4'-\(\alpha\)-\(L\)-rhamnopyranoside (82) using tri-\(O\)-acetyl-1-fluoro-\(\alpha\)-\(L\)-rhamnopyranolsyl (83) as the monosaccharide.\(^{66}\) By this way, they were able to obtain a mixture of 4'-\(O\)-mono and 7,4'-di-\(O\) products in 47\% and 73\% yields respectively. However, this gives a confusing total yield of 120\%, which means that the results are somewhat unreliable. The rest of the publications rely on the use of protecting groups.\(^{149}\) It seems that there is no available method for selective glycosidation at the position 4'-\(O\). All the previously described methods give either mixtures of mono and di products or mixtures of \(\alpha\) and \(\beta\) anomers.

1.4.4 DEUTERATION OF ISOFLAVONES

Among many applications, deuterated (\(\text{D}=^2\text{H}\)) compounds are generally used in the HPLC-MS and GC-MS studies as reference compounds. By deuterating the desired compounds, these will behave in chromatography like their non-deuterated analogues
having the same retention time. However, the difference can be seen in the mass spectrum as they are distinguished by their molecular mass. To obtain mass spectral reliability, there have to be at least three deuterium atoms present in the reference molecule in order to increase the molecular mass above the M+1 and M+2 of the corresponding non-deuterated analogue.\textsuperscript{150} The degree of deuteration is expressed by the term of isotopic purity. It means the percentage of the specific protons exchanged to the deuterons. In the presence of three deuteriums the isotopic purity should be 100\% in order to obtain accurate results in mass spectrometry. In reality, it is normal to introduce four or more deuteriums to the molecule and by doing this to ensure that at least three protons are changed completely. With these properties, the deuterated molecules can be added to a sample and used as internal standards in qualitative and quantitative analysis. Deuterated isoflavones can be divided into two categories according to the method of production. The first method is to introduce the deuterium atoms by total synthesis.\textsuperscript{151} This can be done in a way that starting materials are deuterated which leads to a deuterated product. The only total synthetic route reported for deuterated isoflavones uses a chalcone approach (Scheme 18). Al-Ani \textit{et al.} started the synthesis for 3',5'-dideuteroformononetin (84), which is 4'-O-methylated analogue of daidzein (4), from 3,5-dideutero-4-methoxybenzaldehyde (85).\textsuperscript{151}

\[
\begin{align*}
\text{BnO} & \quad \text{OH} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} \\
\text{O} & \quad \text{Me} & \quad \text{i} & \quad \text{BnO} & \quad \text{OH} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} \\
\text{\textbf{D}} & \quad \text{O} & \quad \text{Me} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}}
\end{align*}
\]

\textbf{(85)}

\[
\begin{align*}
\text{O} & \quad \text{Me} & \quad \text{ii, iii} & \quad \text{O} & \quad \text{Me} & \quad \text{iv} & \quad \text{Bn} & \quad \text{OH} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} \\
\text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} \\
\text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}} & \quad \text{\textbf{D}}
\end{align*}
\]

\textbf{(84)}

\textit{Reagents and conditions}: i) KOH/EtOH, RT ii) pyridine,Ac\textsubscript{2}O iii) Ti(NO\textsubscript{3})\textsubscript{3}-H\textsubscript{2}O,MeOH, KOH, RT iv) cyclohexene, Pd(OH)\textsubscript{2}/C, EtOH, reflux

\textbf{Scheme 18. Synthesis of 3',5'-dideuteroformononetin (84) by chalcone route}

Deuterated compounds can also be obtained by deuterating the target molecule. This can be done by using deuterated acids as reagents, mainly deuterated trifluoro acetic acid (CF\textsubscript{3}COOD) or a phosphoric acid – boron trifluoride – deuterium oxide complex (D\textsubscript{3}PO\textsubscript{4}-BF\textsubscript{3}/D\textsubscript{2}O). The exchange rate of the protons to deuterons follows an order 8>3',5'>6>2',6' for example to daidzein (4).\textsuperscript{152} CF\textsubscript{3}COOD has been used to synthesise 6,8,3',5'-tetraduterodaizdein (86) by refluxing for 9 days\textsuperscript{153} and 6,8,3',5'-tetraduterogenistein (87) by refluxing for 6 days\textsuperscript{154} (Scheme 19).
Scheme 19. Deuteration of isoflavones by CF₃COOD

By the using D₃PO₄-BF₃/D₂O reagent, deuteriums can be introduced even to the less active 2´ and 6´ positions at B-ring.¹⁵⁵ In the case of daidzein (4), to exchange the protons 2´ and 6´ required 7 days in autoclave at 100°C to obtain 6,8,2´,3´,4´,5´-hexadeuterodaidzein (88) (Scheme 20).¹⁵⁶

Scheme 20. Synthesis of 6,8,2´,3´,4´,5´-hexadeuterodaidzein (88) by D₃PO₄-BF₃/D₂O

The D₃PO₄-BF₃/D₂O reagent has also been used in the deuteration of polyhydroxy flavones.¹⁵⁷ Previously flavones have been deuterated with NaOH/D₂O to produce a d₂ or d₃ compound in low isotopic purity.¹⁵₈,¹⁵⁹ As can be seen from the literature, there is a need for a rapid method, producing d₆ deuterated isoflavones in high yield and isotopic purity.

1.4.5 ISOFLAVONES SUBSTITUTED AT POSITION 7-O OR 4´-O

In this chapter, the topics concerning the regioselective O-alkylation and protection of isoflavones at the 7 and 4´ O-positions will be considered. Isoflavones can be O-substituted using typical procedures described to phenols but the case is not always necessarily that straightforward as for phenols owing to many O-positions in isoflavones. Although the selectivity in reactions can be achieved with the aid of the protecting groups, their use can be time consuming. In some cases, protecting groups are essential, like in a
molecule bearing several reactive sites, which differ more or less in their reactivity. However, a selective O-substitution of isoflavones can be simply achieved without the use of protecting groups. Therefore one has to be careful when planning the synthetic route and deciding whether the selective synthesis or use of protecting groups is more advantageous. There are several reports concerning the selective O-substitution of isoflavones. Most of them rely on the acidity difference between phenolic OH groups in isoflavones again, at positions 7 and 4’.

Different computer programmes can be used to estimate the pKₐ values of the OH groups on isoflavones. One of these, Sparc v 3.1, gives the following calculated pKₐ values for OH groups at positions 7 and 4’ in dry DMSO: daidzein (4) 13.87 and 15.64, genistein (5) 10.54 and 12.96, and for glycitein (6) 13.73 and 16.60 respectively. From these values, it can be seen that the acidic difference between the hydroxy groups is almost one hundred fold in all of these compounds. When the calculation is conducted in non-aqueous DMF, the ratio between the pKₐ values of the hydroxy groups stays the same. In genistein (5), the OH group at position 5 is hydrogen bonded to the carbonyl group at position 4, decreasing its reactivity. It is not possible to calculate a pKₐ value for the OH group at position 5 because in the algorithm of these computer programmes the hydrogen bonding option is not included.

Under carefully modified basic conditions, one can introduce an alkyl or acyl group selectively to either positions 7-O or 4’-O, in moderate yields. Common solvents in the substitution reactions of isoflavones include DMF, pyridine, DMSO, acetone, propanol and also aq. KOH. There are numerous bases one can use, but the best one so far, for the regioselective alkylation, seems to be potassium tert-butoxide (t-BuOK) under non-aqueous conditions. This may be due to the fact that t-BuOK is a strong base, capable of deprotonating OH groups on isoflavones, but being bulky at the same time. It has been reported that in some cases the use of an aq. alkaline solution (e.g. NaOH, KOH) may result in the decomposition of the C-ring.

1.4.5.1 CARBOXYLIC ACID CHAIN DERIVATIVES

Carboxylic acid chain derivatives have been synthesised to develop analytical processes and for studying the possible biological activity. As mentioned earlier, the regioselectivity of these following reactions is based on the acidic difference between the OH groups.

The first selective synthesis towards isoflavone carboxylic acid chain derivatives was reported for daidzein (4) in 1989. In this publication, 7-O-ethoxycarbonylpropyldaidzein (89) was synthesised by using t-BuOK as a base in DMF (Scheme 21). The protected carboxylic acid derivative was obtained in 85% yield, after stirring for 10 days at room temperature.
Reagents and conditions: i) t-BuOK, DMF, RT ii) ethyl-4-bromobutyrate

Scheme 21. Selective alkylation of daidzein (4) at position 7-O

By using this method Al-Maharik et al. produced a series of mono-O-alkylated carboxylic acid chain isoflavones, at both 7-O and 4’-O positions in moderate yields (Scheme 22). The common factor of the above reactions is that they are made in two steps in situ, the first step being the formation of the desired potassium phenolate and the second one the addition of the alkyl halide. By controlling the amount of the base (t-BuOK), one can either create mono phenolate (7-OH) or di-phenolate (7-OH and 4’-OH). In the case of diphenolate, the regioselectivity is obtained by the strong nucleophilicity of the 4’-phenolate.

Reagents and conditions: i) 1 eq. t-BuOK, HMPA, 110 °C ii) 2 eq. t-BuOK, DMF, -60 °C iii) 1 eq. ethylbromoacetate

Scheme 22. Selective alkylation of isoflavones at positions 7-O and 4’-O

Wang et al. reported a sonochemical synthesis for synthesising 7-O-ethoxycarbonylmethylgenistein (93) in 85% yield, using K₂CO₃ as a base in acetone. All the previously reported synthetic methods for isoflavone carboxylic acid chain derivatives use halocarboxylic acid ethyl esters as starting materials. After ether
formation, the formed isoflavone carboxylic acid ester is hydrolysed to the corresponding isoflavone carboxylic acids under acidic or basic conditions.\textsuperscript{160,163}

In 2000, Rooke \textit{et al.} introduced a method for synthesising various daidzein-7-\textit{O}-carboxylic acids.\textsuperscript{90} They used halocarboxylic acids as starting materials. It is noteworthy that if the carboxylic acid group is unesterified, the amount of base has to be increased. By using 2 equivalents (eq.) of base, 2 M KOH in acetone, they were able to produce 7-\textit{O}-carboxylic acids in yields varying from 20\% to 55\%. The next year the same group published another study where they synthesised more carboxylic acid chain derivatives.\textsuperscript{166} This time they used free carboxylic acid again but also their ethyl esters. It seemed that there was no remarkable difference in the yields independent whether the carboxylic acid had been protected or not. The yields varied from 9\% to 21\% for the free carboxylic acids, and when ethyl esters were used, the variation was between 2\% and 36\%. No explanations were given for these yield variations. Kohen \textit{et al.} also used unprotected carboxylic acid in the synthesis of 7-\textit{O}-carboxymethyl daidzein (94).\textsuperscript{169} The product was obtained in 16\% yield by refluxing daidzein (4) in n-propanol with sodium n-propylate (made \textit{in situ}) and bromoacetic acid.

1.4.5.2 PROTECTING GROUPS FOR ISOFLAVONES

The introduction of the protecting group to isoflavones can be done by the same methods as used for the \textit{O}-substitution, as discussed in the previous chapter. The common protecting groups used in the protection of isoflavones are benzyl (Bn),\textsuperscript{149,164} \textit{p}-nitrobenzyl,\textsuperscript{146} benzyl ester (Bz),\textsuperscript{164,173} acetyl (Ac),\textsuperscript{108} hexanoyl,\textsuperscript{109,144} allyl\textsuperscript{142,143} and tetrabutylmethylsilyl (TBDMS)\textsuperscript{93,161}. These groups are well described in the literature as protecting groups for phenols.\textsuperscript{174} The most challenging step for isoflavones is, however, deprotection. As already mentioned, isoflavones are protected mainly in the synthesis of glycosides, which cause restrictions for the used cleavage methods. The discussion of the literature concerning the protection is divided into subgroups based on the site of the protection, 7 or 4’ hydroxy group. The deprotection methods are introduced in their own subchapter.

Protection at position 7-\textit{O}

As we know, the hydroxy group at the position 7 is a hundred times more acidic than the OH group at the position 4’. Based on this fact, several different ways to introduce the protecting group selectively to position 7-\textit{O} have been reported. In 1968 Farkas \textit{et al.} synthesised 7-\textit{O}-Bn-genistein (95) using 2 eq. of \textit{K}_2\textit{CO}_3 and 1 eq. of \textit{BnCl} in DMF. They obtained the product in 73\% yield.\textsuperscript{149} Other methods which also rely on the use of relatively mildly basic conditions have been reported, although in lower yields. Nakayma \textit{et al.} introduced synthesis in 1978 for 7-\textit{O}-Bn-daidzein (96), using 2\% aqueous KOH as a base and 1.4 eq. of \textit{BnCl} in DMF/acetone.\textsuperscript{164} The product, which structure was confirmed by \textsuperscript{1}H NMR, was obtained in 45\% yield. In the same publication methods for synthesising 7-\textit{O}-Bz-daidzein (97) and 7-\textit{O}-Bz-genistein (98) were presented.\textsuperscript{164} Instead of KOH in these studies pyridine acts as a solvent and a base. 7-\textit{O}-Bz-daidzein (97) and 7-\textit{O}-Bz-genistein (98) were obtained in 46\% and 58\% yield, respectively.\textsuperscript{164} However, they only reported the UV-VIS spectra and elemental analysis for these compounds. More detailed spectroscopical methods (\textsuperscript{1}H NMR) should be applied as the possibility of
transesterification can not be excluded. This phenomenon will be discussed in the following chapter. In 2003, Jung et al. introduced a total synthetic approach to prepare 7-O-Bn-daidzein (96).\textsuperscript{175} The synthesis started from benzyl protected 2,4-dihydroxyacetophenone (99) and methoxymethoxy protected 4-hydroxybenzaldehyde (100) (Scheme 23). By using the chalcone route they obtained 7-O-Bn-daidzein (96) in 49% yield.\textsuperscript{175}

![Scheme 23. Synthesis of 7-O-Bn-daidzein (96) by the chalcone route](image)

**Reagents and conditions:** i) 9% NaOH, 90% EtOH, 50 °C ii) Tl(NO\textsubscript{3})\textsubscript{3}·3H\textsubscript{2}O, RT iii) 2 M HCl, 50 °C

**Protection at position 4´-O**

There are a few studies describing the protection of isoflavones at position 4´-O. Needs et al. prepared 4´-O-Ac-daidzein (71) by selective hydrolysis of di-O-Ac-daidzein (101).\textsuperscript{108} They used imidazole as a base and obtained the product in 94% yield. Li et al. used a similar approach as they used transesterification to produce 4´-O-hexanoyl daidzein (74) and 4´-O-Ac-daidzein (71) from the corresponding diesters in 94% and 96% yields, respectively.\textsuperscript{144} They also used imidazole as a base and thiophenol (PhSH) as a nucleophile for transesterification in N-methyl-pyrrolidone (NMP). Transesterification has also been used in the synthesis of 4´-O-Bz-genistein (102).\textsuperscript{173} Genistein (5) (1 eq.) was reacted with di-O-Bz-genistein (103) (1 eq.) in pyridine with Ag\textsubscript{2}CO\textsubscript{3} to produce 4´-O-Bz-genistein (102) (2eq.) in 96% yield (Scheme 24). This kind of transesterification reaction is also known in the chemistry of flavones.\textsuperscript{176}
Scheme 24. Transesterification of genistein (5)

In 2003, Fairley et al. introduced a regioselective method for the silylation (TBDMS) of daidzein (4) at position 4'-O by using t-BuOK as a base in DMF in 60% yield.\textsuperscript{93} The same year Szeja et al. synthesised 4'-O-TBDMS-genistein (104) by partial deprotection of the corresponding 7,4'-di-O-TBDMS-genistein (105) with phthalic anhydride in 45% yield.\textsuperscript{161}

Deprotection of ester and ether bonded protecting groups of isoflavones

The deprotection of phenolic protecting groups are well described in the literature.\textsuperscript{174} Usual ways for the deprotection of benzyl protections from phenols are the reductive methods based on the introduction of hydrogen. Protecting groups with an ester linkage are easily hydrolysed under basic and acidic conditions.\textsuperscript{174} The hydrolysis normally leads, in case of isoflavone glycosidation reactions, also to the cleavage of the acetyl groups from the monosaccharide unit.\textsuperscript{108,109,142}

The debenzylation of isoflavones is usually performed with Pd/C, using H\textsubscript{2} or HCOONH\textsubscript{4} as a source of hydrogen. The yields are usually moderate, despite the fact that the reduction of the double bond on C-ring could be suspected. Jung et al. reported that by controlling the hydrogen pressure, debenzylation can be performed selectively.\textsuperscript{175} Under a
hydrogen pressure 1.4 bar (20 psi) they were able to selectively cleave the Bn-group. When the pressure was increased to 2.1 bar (30 psi) the Bn group was cleaved and the double bond was reduced.\textsuperscript{175} The majority of publications, where hydrogenation for debenzylation has been used, do not report the hydrogen pressure or how the pressure is controlled.\textsuperscript{64,149} Al-Ani \textit{et al.} used cyclohexene as a source of hydrogen in the synthesis of 3',5'-dideuteroformononetin (84) in high yield.\textsuperscript{151} Kim \textit{et al.} performed debenzylation for isoflavones with sulphur conjugates at position 2, by using BF$_3$-Et$_2$O-Me$_2$S reagent in dichloromethane.\textsuperscript{59} The reagent was used because of the possible danger for poisoning of palladium catalyst by sulphur. The use of Lewis acids in the debenzylation of phenols is also known in the literature.\textsuperscript{174}

Lewis acids have also been used in the cleavage of allyl group in the presence of acetyl groups. Shozaki used RhCl$_3$ in EtOH to cleave allyl groups from isoflavone acetyl protected glycoside in 73% yield (Scheme 25).\textsuperscript{142,143}

![Scheme 25. Cleavage of allyl protections in the presence of acetyl protected glycoside](image)

\textit{Reagents and conditions}: i) RhCl$_3$, EtOH, reflux

As mentioned earlier, the ester linked protecting groups are easily hydrolysed in basic and acidic conditions. One other option is transesterification. Li \textit{et al.} found out that by using PhSH and imidazole in NMP, they could cleave the hexanoyl protection from 7-OH, selectively, in the presence of benzoyl protection on isoflavone glycoside (Scheme 26).\textsuperscript{144}
Reagents and conditions: i) PhSH, imidazole, NMP, RT

Scheme 26. Cleavage of the hexanoyl group by transesterification

The method is based on the formation of PHS\(^-\) anion, which leads to the transesterification reaction with hexanoyl group.

The removal of the TBDMS group has been performed with 1 M Bu\(_4\)N\(^+\)Br\(^-\) in THF and a 75% aqueous solution of Bu\(_4\)N\(^+\)Br\(^-\) in THF has also been used.\(^{93,161}\)

Lee et al. performed a partial demethylation of isoflavone-C-8-glucoside by using Me\(_3\)SiI in refluxing CH\(_3\)CN.\(^{177}\)

In conclusion, it can be stated that there are only a few regioselective methods for the protection of isoflavones. More work should be done, especially in the field of the protection of the 4\(^-'\) hydroxy and selective deprotection in the presence of sensitive moieties.

1.4.6 CHLORINATION OF ISOFLAVONES

Chlorinated isoflavones and flavones also can be found in cultivated broths of *Streptomyces*.\(^{178,179,180,181}\) *Streptomyces* are bacteria which are mainly found in soil,\(^{182}\) where they take part in the decomposition of organic materials. These bacteria are also
used in pharmaceutical studies to produce medicines, for example antibiotics containing chlorine. In the nature *Streptomyces* are not the only ones responsible of the chlorination of isoflavones. Recently, Kuruto-Niwa *et al.* reported on the possibility of isoflavone chlorination during a water purification process, caused by sodium hypochlorite. As chlorinated isoflavones are formed in the nature, their biological activity should be taken into consideration. It is already known that chlorinated isoflavones possess antioxidant activity.

There are a few synthetic publications among the literature concerning chlorinated isoflavones. Tökes synthesised 8-chloro-3′,4′,5,7-tetrahydroxyisoflavone (106) by starting from 3-chloro-4,6-dimethoxyacetophenone (107) via the chalcone route (Scheme 27).

![Scheme 27. Synthesis of 8-chloro-3′,4′,5,7-tetrahydroxyisoflavone by the chalcone route.](image)

Reagents and conditions: i) 14 M KOH, EtOH, RT ii) Th(NO$_3$)$_3$.3H$_2$O, MeOH, RT iii) 2.8 M HCl, reflux iii) pyridinium hydrochloride, 210 °C

The deoxybenzoin route has been used to produce chlorinated isoflavones, employing the Friedel-Crafts acylation and Houben-Hoesch reaction, from chlorinated starting materials.$^{56,60}$ In some publications chlorinated isoflavones have been obtained via conversion of flavones.$^{73,74,75}$ Recently Kuruto-Niwa *et al.* published a one step method for the synthesis of 6,8-dichlorogenistein (108), 8,3′-dichlorodaidzein (109) and 8,3′,5′-trichlorodaidzein (110) using sodium hypochlorite (NaOCl) with yields varying from 8% to 65%. They were not able to control the chlorination stage. Unidentified mono and di chlorinated isoflavones have been observed in the reaction between isoflavones and hypochlorous acid (HOCl).$^{186,187}$ The use of NaOCl, as well as chlorine (Cl$_2$),$^{188,189,190,191}$ tert-butyl hypochlorite (t-BuOCl),$^{188,192}$ and sulphuryl chloride (SO$_2$Cl$_2$)$^{188,189}$ are common chlorination reagents in the synthesis of chlorinated phenolic compounds. It is known that these reactions often lead to the mixture of chlorinated compounds.
2. AIMS OF THE STUDY

The metabolism and the biological activity of isoflavones in humans are unclear. As we have now learned from the previous chapters, the metabolic route of the isoflavones is complicated and the synthesis of these metabolic compounds is demanding. The aim of this study was to design, synthesise and develop new synthetic methods for human isoflavone metabolites. And by conducting this, to contribute to the research which is carried out in order to understand the role of these compounds in the human body.

In the very beginning of these studies there were no methods available for the direct synthesis of desired metabolic derivatives which include isoflavone sulphates, glucuronides and sulphoglucuronides. The possibility for new applications in the even more expanded area of these synthesised compounds was considered further and hence, it was realised that a rapid and efficient method for the deuteration of isoflavone disulphates is necessary.

From the beginning, it was obvious from the literature that the protecting groups would play an important role in the synthesis of the highly polar isoflavone metabolites. Due to this fact, it was decided to investigate several different methods for the protection. The demands concerning the protection were the regioselectivity of the synthesis and the mild cleavage in the presence of monosaccharide or sulphate moieties.

A part of this study concentrated on the development of a synthetic route towards isoflavone daunorubicin conjugates in collaboration with Professor Skaltsounis from University of Athens, Greece. Daunorubicin (111) is a drug used to prevent the progress of cancer. The basic idea to link daunorubicin (111) with isoflavone was to obtain a molecule, which could overcome the known problem with cancer cells called multi drug resistance (MDR). Different approaches were investigated to obtain this target molecule.

Some interesting observations were noted during the study. One example of these was the chlorination of genistein (5) with thionyl chloride (SOCl₂). This unusual reaction was investigated further, also with other isoflavones, in order to understand the nature of this reaction, and also to develop a synthesis for chlorinated isoflavones that might have antioxidant features.

The results as well as the methods, of these aims are reported in the following chapters.

3. RESULTS AND DISCUSSION

This chapter presents the research work conducted in this study. The aims and how they were reached will be discussed each in their own chapter with conclusions. The methods of the unpublished work will be presented in Chapter 4, Experimental.

3.1 SYNTHESIS OF STARTING MATERIALS

Daidzein (4), dihydrodaidzein (20), dihydrogenistein (21) and equol (19) were synthesised according to the previously published methods. Dihydrogenistein (21) was also synthesised by catalytic hydrogenation (see Chapter 4). Genistein (5) was prepared by
the Houben-Hoesch route and one-pot method.\textsuperscript{60} For glycitein (6) there were only a few published methods, two using chalcone route and one using deoxybenzoin route.\textsuperscript{57,70,89} From the literature it was known that the deoxybenzoins are usually solid, however in the published deoxybenzoin route synthesis for glycitein (6), the corresponding deoxybenzoin was isolated as an oil.\textsuperscript{89} The synthesis of glycitein (6) was started from deoxybenzoic by the Houben-Hoesch route.\textsuperscript{55,80} The starting materials in the synthesis of glycitein (6) by the Houben-Hoesch route are 4-methoxyresorcinol (112) and 4-hydroxyphenylacetonitrile (113) (Scheme 28). 4-Methoxyresorcinol (112) is not a commercially available compound, so it was synthesised from isovanillin (114), as described previously.\textsuperscript{195} The Houben-Hoesch reaction, with 4-methoxyresorcinol (112) and 4-hydroxyphenylacetonitrile (113), gave the desired deoxybenzoin (115) after the hydrolysis step. Ring cyclisation was performed by BF\textsubscript{3}.Et\textsubscript{2}O, MeSO\textsubscript{2}Cl in DMF, first reported by Bass in 1976.\textsuperscript{82} The cyclisation step gave glycitein (6) and partially formylated 7-O-glycitein (116), which was hydrolysed by 0.5M NaOH. After neutralisation, glycitein (6) was obtained as off white solid in moderate yield (see Chapter 4).

Scheme 28. Synthesis of glycitein (6) by the Houben-Hoesch route

The Houben-Hoesch mechanism to deoxybenzoin is considered to start from a reaction between the cyano group and HCl.\textsuperscript{80} Here, the mechanism is proposed for the synthesis of 2,4,4´-trihydroxy-5-methoxydeoxybenzoin (115), starting from 4-hydroxyphenylacetonitrile (113). The formed chloronitrile adduct coordinates to the Lewis acid, ZnCl\textsubscript{2} (Scheme 29). This formed complex then reacts with 4-methoxy-resorcinol (112) by aromatic electrophilic substitution. As seen from the mechanism, the role of ZnCl\textsubscript{2} is only to work as a catalyst, however in practice the amount of ZnCl\textsubscript{2} is nearly at the equivalent level.\textsuperscript{55,57} The formed ketiminium chloride (117) is hydrolysed with acidic H\textsubscript{2}O to give 2,4,4´-trihydroxy-5-methoxydeoxybenzoin (115).
Scheme 29. Proposed mechanism for synthesis of 2,4,4'-trihydroxy-5-methoxydeoxybenzoin (115) by the Houben-Hoesch reaction
3.2 SYNTHESIS OF ISOFLAVONOID DISULPHATES

The goal was to develop a method for the sulphation of various polyhydroxy isoflavonoids. These isoflavonoid O-sulphates, which are also human metabolites, could be used in the research of isoflavone metabolism and to contribute in understanding the biological activity of isoflavonoid O-sulphates.

All the previously published sulphation methods have yielded mixtures of isoflavone mono and disulphates. As we know, there are multiple ways to introduce SO₃ into the OH group of phenol (See Chapter 1.4.2). Only few of these methods fulfilled our criteria which were selectivity of the reaction, simple procedure and work up. One critical aspect was the solubility of the starting isoflavonoids. From the previous experiences it was known that solvents, such as DMF and pyridine dissolve isoflavones well. Both of these solvents are known to form complexes with SO₃ which are then used in the synthesis of sulphate esters (See Chapter 1.4.2). The sulphation of isoflavones was started with a SO₃-pyridine complex (52) made in situ. The reaction conditions were first optimised for daidzein (4) since its disulphate has been isolated from rat urine (Scheme 31). Fairley et al. also used SO₃ pyridine complex (52) in their method to produce daidzein di and mono sulphates (11, 14, 18). The sulphation reaction proceeded well with daidzein (4) but the isolation of the formed daidzein-7,4´-di-O-sulphate (18) seemed to be a problem. As seen in the Scheme 30, the formed sulphate ester forms a salt with protonated pyridine.

![Scheme 30. Sulphation of phenol by the pyridine sulphur trioxide complex](image)

It is common to use inorganic bases in order to release the sulphate ester from its pyridine salt. Sulphate ester is then isolated as a potassium or sodium salt. From the ¹H NMR it was clear that the sulphation of daidzein (4) had taken place (seen as a shift of the aromatic protons ortho to phenol) and that no by-products were present. The only impurities were the salt residues of the used base (NaHCO₃) in the isolation step and traces of pyridine. Options for the purification due to the highly polar nature of the daidzein-7,4´-di-O-sulphate (18) disodium salt were reverse phase (RP-18) and gel filtration, also known as size exclusion chromatography. Recrystallisation was also investigated, but due to the high amount of salts and the solubility of the formed sulphate in water, these attempts were unsuccessful. The use of reverse phase chromatography, solid phase extraction or preparative RP-18 TLC did not lead to pure compounds. It was decided to try gel filtration. The optimal gel was found to be Sephadex LH-20. Water was chosen as an eluent in the gel filtration process, as the formed daidzein-7,4´-di-O-sulphate (18) disodium salt was readily soluble in water. The retention of the daidzein-7,4´-di-O-sulphate (18) disodium salt was followed by RP-18 plates eluting with MeOH:H₂O (4 : 1). Evaporation of the combined fractions, containing the target molecule, gave the desired compound in moderate yield. This presented reaction method was applied to genistein (5). It was observed that the hydrogen bond between the OH at position 5 and carbonyl at position 4 in genistein (5) was strong enough to inhibit sulphation at position 5. Thus no
protection was needed to obtain the genistein-7,4′-di-O-sulphate (27) disodium salt. As a result of these successful results with daidzein (4) and genistein (5), the sulphation method was introduced also to minor isoflavone metabolites that could form disulphates in a human. These were glycitein (6) (Scheme 31), dihydrodaidzein (20), dihydrogenistein (21) (Scheme 32) and equol (19) (Scheme 33). All of the above compounds gave their corresponding disulphates in moderate yields (I).

Reagents and conditions: i) ClSO₃H, pyridine, -16 ° to RT ii) 5% NaHCO₃, RT

Scheme 31. Synthesis of isoflavone-di-O-sulphate disodium salts

Reagents and conditions: i) ClSO₃H, pyridine, -16 ° to RT ii) 5% NaHCO₃, RT

Scheme 32. Synthesis of isoflavonone-di-O-sulphate disodium salts

Reagents and conditions: i) ClSO₃H, pyridine, -16 ° to RT ii) 5% NaHCO₃, RT

Scheme 33. Synthesis of equol-di-O-sulphate disodium salt
Another approach investigated to access di-O-sulphated isoflavonoids was the use of a solid supported synthesis in which a polymer bound SO\textsubscript{3}pyridine complex is used as a sulphation agent. This method should provide an easy purification step by simple filtration of the used reagent. However, in our experience no sulphation was observed at all. This may be due to the steric hindrance of the polymer chain. The commercially available DMF\textsubscript{3}SO\textsubscript{3} and pyridine SO\textsubscript{3} complexes were also examined. The results, compared to the \textit{in situ} prepared pyridine SO\textsubscript{3} complex (52), were not satisfying. Only partial sulphation was observed with these commercially available complexes. The TCE protected sulphation method was not tested in this case as the required hydrogenolysis step had been seen to lead to the partial reduction of the double bond on C-ring.

### 3.3 SYNTHESIS OF DEUTERATED ISOFLAVONE DISULPHATES

Deuterated isoflavones can be used in HPLC-MS studies of isoflavones and their metabolites. Isotopically labelled di-O-sulphated isoflavone derivatives are useful tools for further understanding the biological activity as well as their behaviour in the analysis and isolation process of the biological samples. These labelled di-O-sulphated isoflavones could be used as internal standards in physiological samples in order to investigate their chemical stability as well as the obtained yield after the isolation from the samples and hence determine the accuracy of the analysis results.

The aim was to develop a low cost, rapid and clean synthesis towards deuterated isoflavone disulphates. The reasons for the deuteration to be carried out before the formation of the sulphate ester are the possible hydrolysis under highly acidic conditions and the low solubility of the corresponding disodium salt. As known from the literature, CF\textsubscript{3}COOD is capable of deuterating daidzein (4) to give 6,8,3′,5′-tetradeuterodaidzein (86) when refluxed for 9 days. It was also known that the use of D\textsubscript{3}PO\textsubscript{4}-BF\textsubscript{3}/D\textsubscript{2}O under high temperature and pressure is required to obtain 6,8,2′,3′,5′,6′-hexadeuterodaidzein (88). Since the handling and preparing of the D\textsubscript{3}PO\textsubscript{4}-BF\textsubscript{3}/D\textsubscript{2}O reagent is uncomfortable, giving moderate yields, it was decided to use CF\textsubscript{3}COOD as a deuterating agent instead. Isoflavones were predeuterated by evaporation from D\textsubscript{2}O/acetone solution to exchange the phenolic protons to deuteriums. The deuteration of the aromatic protons was performed under microwave irradiation in order to obtain the required temperature and pressure for the reaction. With this approach, all three isoflavones were obtained as \textit{d\textsubscript{6}} deuterated analogues with high isotopic purity and high yields after the recrystallisation step. In genistein-\textit{d\textsubscript{6}} (121), the deuterium atoms at C-6 and C-8 are labile, and hence the back change was done by refluxing 6,8,2′,3′,5′,6′-hexadeuterogenistein (121) in a solution of 0.5% CH\textsubscript{3}COCl/MeOH for 10 min to give 2′,3′,5′,6′-tetradeuterogenistein (122). The identity and the purity of the compounds were verified by HPLC-MS (ESI+), \textsuperscript{1}H and \textsuperscript{13}C NMR techniques. Only traces of M-1 were detected in ESI+ leading to an isotopic purity over 90%. The sulphation of these compounds was performed as described previously in the Chapter 3.1. All the deuterated analogues were stable under these reaction conditions and the desired deuterated isoflavone disulphates were obtained in moderate yield (Scheme 34) (II). The isotopic purity of the compounds were confirmed by ESI- and \textsuperscript{1}H and \textsuperscript{13}C NMR analysis.
**Reagents and conditions:**
i. D$_2$O/acetone, +40 °C
ii. CF$_3$COOD, MW
iii. 0.5% CH$_3$COCl/MeOH, reflux
iv. ClSO$_3$H, pyridine, -16 °C

**Scheme 34.** Synthesis of deuterated isoflavone-di-O-sulphate disodium salts

**3.4 SYNTHESIS OF DAIDZEIN-7-O-β-D-GLUCURONIDE-4’-O-SULPHATE**

Daidzein-7-O-β-D-glucuronide-4’-O-sulphate (16) is one of the known isoflavone human metabolites. However, the biological activity of this compound in human body is still unclear. To confirm the structure of the isolated analogue and to provide a tool for the study of the biological activity of this compound, daidzein-7-O-β-D-glucuronide-4’-O-sulphate (16) was synthesised.

A selective glucuronation at position 7-OH was explored according to the earlier reported method for isoflavone-7-O-glucosides. This, however, did not give the expected acetyl glucuronide conjugate. From this, and previous observations, it was clear that the protection was indeed needed for a reaction of this kind. Hence, it was decided to investigate the use of benzyl protection at the position 4’-OH, due to its stability under the used reaction conditions, and also due to its selective cleavage. TBDMS protection was also tested but the cleavage resulted to be very problematic and no expected product could be isolated. The glucuronidation at the position 7-OH was performed with the two-phase reaction described earlier for phenols, and also by the Koenigs-Knorr reaction which has been reported for daidzein (4). When comparing these two methods, there was no
notable difference in the yields, but due to its simpler performance the two-phase reaction was selected.

Debenzylation of the daidzein-4′-O-Bn-7-O-triacetylglucuronic acid methyl ester (127) was first carried out with Pd/C and H₂, but it resulted in a partial reduction of the double bond in the C-ring. The use of HCOONH₄, instead of H₂, as source of hydrogen did not solve the problem. This undesired reaction was prevented by using earlier reported debenzylation for tyrosine with thioanisole/TFA (Scheme 35). By using this successful ‘push and pull’ method for the debenzylation, all the acetyl groups as well as the methyl ester were stable under these conditions.

Scheme 35. Debenzylation of (127) by the “push and pull” method

The sulphation of the daidzein-7-O-triacetylglucuronic acid methyl ester (128) was successfully performed with pyridine-SO₃ complex made in situ, as reported earlier in Chapter 3.1 for isoflavonoid di-O-sulphates. The hydrolysis of the acetyl groups and the methyl ester was first carried out with 1M NaOH in acetone. Probably due to the low solubility of the (129), the hydrolysis resulted in an unidentified mixture of products. The sulphation step and the hydrolysis of the acetyl groups and the methyl ester were carried out in situ to give daidzein-7-O-β-D-glucuronide-4′-O-sulfate (16) disodium salt in 41% yield (Scheme 36) (III).
Scheme 36. Synthesis of daidzein-7-O-glucuronide-4′-O-sulphate disodium salt (16)

3.5 SYNTHESIS OF CHLORINATED ISOFлавONES

Chlorinated isoflavones and flavones have been found in the cultivated broths of *Streptomyces*. The common factor of these isolated chlorinated isoflavones is that they all have a hydrogen bonded OH group at the position 5 (Figure 9).
Figure 9. Isolated isoflavone chlorides from cultivated broths

The synthetic methods for these chlorinated compounds have mainly been based on a total synthesis or electrophilic aromatic substitution assisted by Cl$^+$. A selective chlorination of genistein (5) was observed during the synthesis of genistein acyl chloride derivative by thionyl chloride and it was decided to further investigate this fascinating reaction. This observation could lead to an alternative way to synthesise selectively chlorinated isoflavones.

First, a series of reactions was made with isoflavones to observe their behaviour in neat thionyl chloride. The reactions with daidzein (4) and glycine (6) gave unidentified mixtures of chlorinated compounds while genistein (5) and biochanin A (134) behaved completely differently, since selectively chlorinated compounds were isolated. A common factor of genistein (5) and biochanin A (134) is the hydrogen bonded hydroxy group at position 5 capable for intramolecular hydrogen bond with C=O group. In nature, this hydrogen bonded hydroxy group can be seen as an essential property between the chlorinated isoflavones, isolated from cultivated broths (Figure 9).

To rule out the possibility that chlorination is a consequence of a reaction between thionyl chloride and the OH groups, genistein (5) was protected with methyl iodide (MeI) to give 5,7,4'-trimethoxy-genistein (135). It seemed, according to HPLC-MS, that the chlorination proceeded in spite of the methylation, although more slowly than with genistein (5) and without selectivity. The loss of the selectivity after the methylation of the 5-OH may be a consequence of the absence of the hydrogen bond between hydroxy group at position 5 and carbonyl at position 4. Methylation, however, does not prevent the participation of the free electron pair of the oxygen at position 5, which might be the key factor in the reaction (Scheme 37).
Further experiments were conducted with resorcinol, phloroglucinol and trihydroxyacetophenone. A mixture of various unidentified chlorinated products were obtained without any selectivity.

The reaction between genistein (5) and SOCl$_2$ was investigated with HPLC-MS and $^1$H NMR. It was observed that the reaction proceeded first to the 8-chlorogenistein (131) (Scheme 38). Neither prolongation of the reaction time nor heating of the reaction mixture lead to 6,8-dichlorogenistein (108). Since it is a well-known fact that the reactions with SOCl$_2$ can be catalysed by a tertiary amine, in our case we investigated the effect of Et$_3$N. From these results it was seen that the presence of a catalytic amount of Et$_3$N slowed the reaction notably. In addition, extra signals in the $^1$H NMR spectrum indicated a reaction between SOCl$_2$ and Et$_3$N. This was further confirmed by conducting the reaction only with SOCl$_2$ and Et$_3$N yielding the same signals in the $^1$H NMR spectrum.

Moore et al. studied the chlorination of 4α-methyl-1,3,9-triphenyl-4αH-fluorene using thionyl chloride and dimethyl sulphoxide. We also applied this method in our reaction with genistein (5) by varying the concentration of DMSO. The reaction indeed proceeded, but gave various chlorinated products. These products were not isolated or analysed since the desired selectivity of the chlorination was not obtained. In their article Moore et al. also proposed the precursor of the α-chlorosulfide which is formed from the reaction between sulphoxide and thionyl chloride. This precursor is well known from the Swern oxidation reaction where DMSO is activated with oxalyl chloride.

6,8-Dichlorogenistein (108) was obtained in moderate yield by repeated evaporation and addition of the fresh SOCl$_2$, (Scheme 38) (IV). Interestingly, with biochanin A (134), only dichlorinated product (136) could be isolated.
Reagents and conditions: i) SOCl₂, RT ii) SOCl₂, evaporated and repeated

Scheme 38. Synthesis of isoflavone chlorides

In the HMBC ($^1$H-$^{13}$C) spectrum of (131), couplings between the proton H-6 and carbons C-8, C-4a, C-5, C-7 were clearly seen, again indicating the chlorination at C-8 position (Scheme 38). In the $^{13}$C NMR spectra of 8-chlorogenistein (131) and 6, 8-dichlorogenistein (108), the effect of the chlorine atom as an electron-withdrawing group was clearly seen from the shifting of the signals. The carbons ortho to the chlorine atom experienced a decrease in electron density resulting in deshielding by 4.0-4.5 ppm. On the other hand, the carbon bearing the chlorine atom showed an increase in electron density resulting in shielding by 3.6-5.1 ppm. In both cases, EI mass spectra indicated that the chlorine atom(s) are located on A-ring. For 8-chlorogenistein (131), clear peaks at 304 m/z (M+), 186 m/z (A-ring) and 118 m/z (B-ring) were detected whereas for 6,8-dichlorogenistein (108), the peaks at 338 m/z (M+), 220 m/z (A-ring) and 118 m/z (B-ring) were observed. Similarly for 6,8-dichlorobiochanin A (136) peaks at 352 m/z (M+), 220 m/z (A-ring) and 132 m/z (B-ring) were observed. Peaks with higher m/z are the molecular ion of the compound concerned and the lower m/z peaks are the result of the retro Diels-Alder fragmentation patterns respectively. The previously published mass spectra of 8-chloro-3’,4’,5,7-tetrahydroxyisoflavone (106) show similar fragmentation patterns as obtained for our compounds. The $^{13}$C NMR spectra were compared with the previously published spectra of isolated genistein chlorine adducts. From the previously published spectrum it was clearly seen that in the case of 6,8-dichlorogenistein (108), the $^{13}$C NMR spectrum was incomplete, missing a significant amount of signals. Anyanwutaku et al. reported only 8 signals whereas from our spectrum, the total of 13 signals could be assigned, otherwise the spectral data were in agreement with ours.
No mechanism for this type of reaction is known in the literature. Indeed, few publications can be found suggesting mechanistic approaches for chlorination of this kind, mainly concentrating on the repeated pathways. Carre et al. reported the following reaction equation for chlorination of phenols. It is based on the reaction of three equivalent of phenol and two equivalent of SOCl₂ producing 1,1',1''-(chloro-λ⁴-sulphanetriyl)tribenzene (137), 3 C₆H₅OH + 2 SOCl₂ = (OHC₆H₄)₃SCl + SO₂ + 3 HCl. The formed 1,1',1''-(chloro-λ⁴-sulphanetriyl)tribenzene (137) readily decomposes as follows, (OHC₆H₄)₃SCl = (OHC₆H₄)S + OHCl. When this equation is considered from a mechanistic point of view, the following suggestion is presented (Scheme 39).

Scheme 39. Proposed mechanism for the reaction between phenol and SOCl₂
This mechanistic approach is convenient when dealing with small molecules as the formation of 1,1',1''-(chloro-\(\lambda^4\)-sulphanetriyl)tribenzene (137) species is sterically possible after repeated reactions of phenol at the sulphur of \(\text{SOCl}_2\). However, in our case, where the sterically hindered molecule is concerned, a different approach for the mechanism has to be applied.

Bissinger et al. studied the reactions of phenols with \(\text{SOCl}_2\), trying to produce phenyl chlorosulphinate (138).\(^{204}\) They reported that when using 2 eq. of the phenol with 1 eq. of \(\text{SOCl}_2\), traces of \(p\)-chlorophenol was obtained instead of the desired product (Scheme 40).

\[
\begin{align*}
\text{HO} & \xrightarrow{\text{OH}} \text{Cl} \\
\text{HO} & \xrightarrow{\text{ClSO}} \text{S} \text{Cl} \\
\end{align*}
\]

\textbf{Scheme 40.} Reaction between phenol and \(\text{SOCl}_2\) resulting in \(p\)-chlorophenol

This is clear evidence from their report that the reaction is not taking place at the phenolic hydroxyl group excluding this approach from our mechanism. The two options for the mechanism are proposed, both based on aromatic electrophilic substitution. Both ways lead also to the excretion of sulphur monoxide.\(^{205}\) The first one is electrophilic substitution to the sulphur at \(\text{SOCl}_2\) (Scheme 41). The other one is the attack of the aromatic ring directly to the chlorine in \(\text{SOCl}_2\) followed by the expulsion of \(\text{SOCl}\).
Scheme 41. Proposed mechanism for the chlorination of genistein (5) by SOCl₂
3.6 SELECTIVE SYNTHESIS OF ISOFLAVONE O-CONJUGATES

This chapter concentrates on the selective synthesis of various mono-O-conjugated isoflavones. These include protected isoflavones as well as carboxylic acid chain conjugates. Protecting groups were studied in connection with the synthesis of isoflavone glucoside and glucuronide conjugates. Isoflavone carboxylic acid chain derivatives were synthesised for two different purposes. The first was to synthesise glycine carboxymethyl conjugates, which could be used in the production of glycine antibodies in the development time resolved fluorescent immunoassay (TR-FIA) for glycine (6). TR-FIA is a sensitive method for detecting isoflavones from biological samples on a nanomolar (nmol/l) scale.206,207 This method has already been used with other isoflavones, daidzein (4) and genistein (5).169,206,207 The second was to use carboxylic acid chain as a linker between isoflavone and daunorubicin (111) to produce a molecule which could be used in the study of MDR (Scheme 50).

3.6.1 REGIOSELECTIVE PROTECTION AND DEPROTECTION OF ISOFLAVONES

Different protecting groups for phenolic OH-groups at isoflavones were investigated during this study. The protecting groups were chosen on the basis of their stability under basic conditions. The cleavage of these protecting groups in the presence of acid sensitive substituents, such as sulphate or monosachharide moieties was also a critical aspect. Deprotection should also be possible to be performed selectively in the presence of other protecting groups, for example cleavage of the benzyl protection in the presence of acetyl protection.

The following protecting groups were investigated: benzyl (Bn), benzyloxy (Bz), acetyl (Ac) and t-butyldimethylsilyl (TBDMS).93 All these protecting groups can be introduced to isoflavones regioselectively using t-BuOK as a base (see Chapter 4) (Scheme 42). These protected isoflavones are useful in the reactions where a direct selective introduction of the desired group is not possible. The use of these protecting groups enables the production of selectively conjugated isoflavones.
Reagents and conditions: i) 1 eq. t-BuOK, DMF, RT ii) 3 eq. t-BuOK, DMF, RT iii) 1 eq. corresponding halide, RT

Scheme 42. Synthesis of regioselectively protected isoflavones

Introduction of the Bn group in total synthesis was also studied. In our experience, the Bn group cannot be introduced at the beginning of the isoflavone synthesis when using the Houben-Hoesch or Friedel-Crafts type approaches, as the reaction conditions in both of these reaction routes are highly acidic. In a Houben-Hoesch reaction hydrogen chloride is introduced to the reaction mixture and in a Friedel-Crafts acylation hydrogen fluoride is formed during the reaction. Partial cleavage is observed in both of these reactions, regardless of the position of the Bn group (7-O or 4′-O).

Transesterification was also employed in the synthesis of Bz protected isoflavones. This known phenomenon (see Chapter 1.4.5.2) was first observed in the synthesis of isoflavone 4′-O-glucosides by a two-phase reaction. It was noticed that the use of Bz protection in the position 7-O in the synthesis of 4′-O-glucoside resulted in the transition of the Bz group to position 4′-O and the glycosidation to position 7-O. This observation was confirmed by conducting the reaction for only 7-O-Bz-daidzein (97) under the same two-phase
conditions. The result was that only 4’-O-Bz-daidzein (144) and daidzein (4) could be isolated. It seems that the transesterification is rapidly taking place since no 4’-O glycosidated compounds were detected in the glycosidation reaction. Further studies were conducted with different bases. By using t-BuOK (1 eq.) as a base in DMF, 7-O-Bz-daidzein (97) could be transformed into 4’-O-Bz-daidzein (144) in 40% yield. Despite the anhydrous conditions, a partial hydrolysis was obtained. Obviously, traces of H₂O were present. The same results were obtained with genistein (5) and glycitein (6). It was also noted that by using K₂CO₃ (5 eq.) as a base in DMF, 4’-O-Bz-isoflavones could be synthesised with moderate yields with BzCl (see Chapter 4) (Scheme 43).

![Diagram](image)

Reagents and conditions: i) K₂CO₃, DMF, RT ii) BzCl, RT

Scheme 43. Synthesis of 4’-O-Bz-isoflavones by using K₂CO₃

Selective insertion of the protecting groups was achieved successfully but the deprotection appeared to be problematic. The ester bonded protecting groups were easily hydrolysed using KOH in EtOH but the cleavage of the phenolic Bn group by common methods like Pd/C and H₂ or HCOONH₄ resulted in a partial reduction of the double bond in the C-ring. This problem was finally overcome using the ‘push and pull’ method, described in the Chapter 3.4.

3.6.2 SYNTHESIS OF GLYCITEIN CARBOXYMETHYL O-CONJUGATES

As mentioned in the introduction part of this chapter, carboxylic acid derivatives were needed in two cases, for the development of TR-FIA for glycitein (6) and the synthesis of isoflavone daunorubicin conjugates.

The glycitein carboxymethyl O-conjugates were synthesised by previously published methods for daidzein (4) and genistein (5) with slight variations. The regioselective reaction was achieved by controlling the amount of the base and using DMF as a solvent at room temperature. Both 7 and 4’ O-conjugates (146, 147) were obtained in moderate yields after the hydrolysis step (see Chapter 4) (Scheme 44). The problematic step in the synthesis was the purification of the formed carboxymethyl ester conjugates. Due to the low solubility of glycitein (6), compared to that of daidzein (4) and genistein (5), some product was repeatedly lost in the purification step (compared to HPLC).
Reagents and conditions: i) 1 eq. t-BuOK, DMF, RT ii) ethylbromoacetate, RT iii) 3 eq. t-BuOK, RT iv) ethylbromoacetate, RT v) COOH, p-toluenesulphonic acid, reflux

Scheme 44. Synthesis of glycine carboxymethyl derivatives

3.6.3 SYNTHESIS OF DAIDZEIN-4'-O-β-GLUCOSIDE

In 1976, daidzein-4'-O-β-glucoside (78) was isolated from *Piptanthus nepalensis* by Paris *et al.* Lewis *et al.* reported in 1998 that they had developed a method for regioselective synthesis for daidzein and genistein 4'-O-β-glucosides (78, 77). In 2001, Boryski *et al.* reported that they had noticed that in the spectrum of genistein-4'-O-β-glucoside (77), published by Lewis *et al.*, the diagnostic signals of the remaining phenolic OH at 4' (9.60 ppm), and aromatic protons 3', 5'-H (6.83 ppm), 6-H (6.47 ppm) and 8-H (6.72 ppm) confirmed a substitution to position 7-O instead of claimed position 4'-O (Table 2).

There are two publications which report the NMR spectra of isolated genistein-4'-O-β-glucoside (77). When the spectrum of the synthesised genistein-4'-O-β-glucoside (77) is compared to the spectrum of isolated genistein-7-O-β-glucoside (2), the spectra are virtually identical (Table 2).
Table 2. $^1$H NMR comparison of genistein (5), genistin (2) and sophoricoside (77)

<table>
<thead>
<tr>
<th>$^1$H</th>
<th>genistein (5)</th>
<th>genistin (2)</th>
<th>genistin (2)</th>
<th>(77)</th>
<th>(77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>isolated</td>
<td>synthesised</td>
<td>isolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(210)</td>
<td>(139)</td>
<td>(209)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-2</td>
<td>8.32</td>
<td>8.34</td>
<td>8.35</td>
<td>8.37</td>
<td>8.43</td>
</tr>
<tr>
<td>H-5</td>
<td>12.96 (OH)</td>
<td>-</td>
<td>12.93 (OH)</td>
<td>12.90 (OH)</td>
<td>12.94 (OH)</td>
</tr>
<tr>
<td>H-6</td>
<td>6.23</td>
<td>6.47</td>
<td>6.55</td>
<td>6.23</td>
<td>6.47</td>
</tr>
<tr>
<td>H-8</td>
<td>6.39</td>
<td>6.73</td>
<td>6.79</td>
<td>6.39</td>
<td>6.72</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>7.38</td>
<td>7.41</td>
<td>7.44</td>
<td>7.49</td>
<td>7.40</td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>6.83</td>
<td>6.83</td>
<td>6.91</td>
<td>7.09</td>
<td>6.83</td>
</tr>
</tbody>
</table>

$\delta$ values in ppm, solvent DMSO-$d_6$

From these observations it was obvious that the synthesis described to genistein-4′-O-β-glucoside (77) in fact yields the corresponding 7-O analogue (2). In 2006, Weis et al. biosynthesised daidzein-4′-O-β-glucoside (78), which was characterised by $^1$H NMR. Due to the NMR solvent they used (CD$_3$OD), the comparison of the spectrum with the synthesised analogue is rather complicated (Table 3). To confirm the structure of daidzein-4′-O-β-glucoside (78), it was decided to synthesise by using a protecting group to obtain the absolute stereochemistry, in this case β anomer.

Table 3. $^1$H NMR comparison of daidzein (4) and daizein-4′-O-glucoside (78)

<table>
<thead>
<tr>
<th>$^1$H</th>
<th>daidzein (4) a)</th>
<th>(78) synthesised $^{147}$ a)</th>
<th>(78) entsymatic $^{211}$ b)</th>
<th>(78) a), c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(78)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-2</td>
<td>8.29</td>
<td>8.43</td>
<td>8.17</td>
<td>8.35</td>
</tr>
<tr>
<td>H-5</td>
<td>7.96</td>
<td>8.05</td>
<td>8.06</td>
<td>7.97</td>
</tr>
<tr>
<td>H-6</td>
<td>6.93</td>
<td>7.02</td>
<td>6.94</td>
<td>6.94</td>
</tr>
<tr>
<td>H-8</td>
<td>6.86</td>
<td>6.96</td>
<td>7.16</td>
<td>6.87</td>
</tr>
<tr>
<td>H-2', 6'</td>
<td>7.38</td>
<td>7.58</td>
<td>7.49</td>
<td>7.50</td>
</tr>
<tr>
<td>H-3', 5'</td>
<td>6.80</td>
<td>7.16</td>
<td>6.86</td>
<td>7.08</td>
</tr>
</tbody>
</table>

$\delta$ values in ppm, a) DMSO-$d_6$, b) CD$_3$OD, c) see chapter 4

The initial approach was first to protect position 7-O of daidzein (4) with Ac and Bz groups. Glycosidation was attempted with the Koenigs-Knorr method and also with a two-phase method, described in connection with sulphoglucuronide synthesis (Chapter 3.4). Both of these glycosidation methods resulted in transesterification of Ac and Bz groups. The protecting group was changed to Bn, since it was known to be stable under the these glycosidation conditions (Chapter 3.4). After the successful benzylaion of position 7-O, glycosidation to position 4′-O was performed by the two-phase reaction with 32% yield. After debenzylation by thioanisole/TFA and ester hydrolysis by KOH in EtOH, the target compound daidzein-4′-O-β-glucoside (78) was obtained (see Chapter 4) (Scheme 45).
Reagents and conditions: i) t-BuOK, DMF, RT ii) BnCl. iii) α-acetobromoglucose, K₂CO₃, Bu₄NBr, CHCl₃, H₂O, RT iv) thioanisole, TFA, 40 °C v) KOH, EtOH, RT

Scheme 45. Synthesis of daidzein-4′-O-β-glucoside (78)

The structure of the compound was verified with ¹H and ¹³C NMR and 2D NMR [HMQC (¹H-¹³C), HMBC (¹H-¹³C)]. When comparing these spectra (¹H and ¹³C NMR) with the spectrum of previously synthesised (78),¹⁴⁷ it can be seen that the diagnostic signals correlate (Table 3). However, the synthesis of daidzein-4′-O-β-glucoside (78) described here gives the absolute conformation including the assignments of the hydroxy groups at monosaccharide moiety.

3.6.4 SYNTHESIS OF DAIDZEIN-4′-O-Bz-7-O-TRIACETYLGLUCURONIDE METHYL ESTER

As previously mentioned, the transesterification between the hydroxy groups 7 and 4′ can be exploited in the synthesis of 7-O-glycosides (Chapter 3.6.1). This phenomenon was observed during the synthesis of daidzein-4′-O-β-glucoside (78). The Bz protection at
position 7-\textit{O} was transferred during the synthesis to position 4'-\textit{O} and the glycosidation took place at position 7-\textit{O}. This observation encouraged us to try this approach in the synthesis of daidzein-4'-\textit{O}-Bz-7-\textit{O}-triacetylglucuronide methyl ester (150), which could be then hydrolysed to give daizein-7-\textit{O}-\textbeta-gluconurone (8). The synthesis was started from daidzein (4) by selective benzoylation at the position 7-\textit{O} (Scheme 46). Glycosidation was performed by the earlier described two-phase reaction, during which the transesterification took place. The target molecule (150) was obtained, after purification, in 34\% yield (see Chapter 4).

\begin{center}
\textbf{Scheme 46. Synthesis of daidzein-4'-O-Bz-7-O-triacetylglucuronide methyl ester (150)}
\end{center}

The main advantages of this approach are the clean reaction and easy purification, since the formed product is readily soluble in organic halogenated solvents affording liquid extraction steps, followed by column chromatography and recrystallisation. It has to be noted that in the situation where the hydroxy group at position 4' is unprotected and the protecting groups at glucuronic acid are still present, the molecule is polar making the purification more complex by column chromatography. In our experience, only recrystallisation can be used (see Chapter 3.4). As can be seen, protecting groups play an important role in the synthesis of isoflavone glucosides and glucuronides, regarding both selectivity and solubility.
3.6.5 SYNTHESIS OF DAUNORUBICIN CONJUGATE OF BIOCHANIN A

There are few publications concerning the coupling of daunorubicin (111) to estrogen type of compounds, which might enhance the selectivity of the antitumor agents against tumor cells or tissues.\textsuperscript{212,213} The idea is that a carrier molecule which has shown selectivity towards the desired binding site will target the cytotoxicity of daunorubicin (111) selectively to the tumors. This carrier type of approach could also be used to overcome the known multi drug resistance (MDR) problem.

Biochanin A (134) which itself has shown some biological activity in human was chosen for a carrier molecule. The first option for the linker between biochanin A (134) and daunorubicin (111) was an alkylamine chain (Scheme 47). The problem, however, was the cleavage of the phthalimide protection. The use of hydrazine hydrate resulted in a reaction at the C-ring leading to compound (152) (see Chapter 4) (Scheme 47).

![Scheme 47. Synthetic approach to an alkylamine derivative of biochanin A](image)

Reagents and conditions: i) \(\text{N-(4-bromobutyl)phthalimide, } \text{K}_2\text{CO}_3, \text{NaI, acetone, reflux}\)

\(\text{ii) } \text{N}_2\text{H}_4\text{H}_2\text{O, EtOH, reflux}\)

This is a known behaviour of isoflavones and benzyl flavones under these reaction conditions.\textsuperscript{214,215} The following mechanism is based on the mechanism suggested by Lévali et al. for 3-benzylchromones (Scheme 48).\textsuperscript{214}
Scheme 48. Proposed mechanism for pyrazole formation by hydrazine hydrate

Hydrolysis of the phthalimido moiety was also studied with NaOH, HCl and MeNH₂ without any success. There are different approaches to obtain the amine group, including the reduction of the nitrile (CN) or nitro (NO₂) functionalities. However, the low reactivity of these alkyl halides and the potential reduction of the double bond at C-ring forced us to consider other approaches. It was decided to use carboxylic acid functionality at the point of attachment, due to the possibilities of activation procedures that can be applied. The options for the activation of carboxylic acid were: formation of the acid chloride, use of DCC as an activator or use of mixed anhydride. The formation of the acid chloride resulted in the chlorination of biochanin A (see Chapter 3.5). The problem in the DCC activation was that instead of the desired reaction, the O-acyl urea decomposed to the corresponding amide derivative (Scheme 49). This is a well-known problem when dealing with poor nucleophiles.

Scheme 49. Decomposition of O-acyl urea

The mixed anhydride method, using pivaloyl chloride worked successfully giving the desired product, daunorubicin biochanin A derivative (153) (see Chapter 4) (Scheme 50). Similar kind of synthesis using mixed anhydride method has been described for the synthesis of a daunorubicin estrogen conjugate. The biological activity of (153) was tested on MCF7 and Ishikawa cell lines. The compound (153) did not show any activity on these cell lines. Other potential cell lines will be tested in the future.
Reagents and conditions: i) ethyl 4-bromo butyrate, K₂CO₃, KI, acetone, reflux ii) HCOOH, p-toluene-sulphonic acid, H₂O, reflux iii) pivaloyl chloride, Et₃N, DMF, -16 °C iv) Et₃N, DMF, -16 °C to RT

Scheme 50. Synthesis of the daunorubicin biochanin A derivative (153)

4. EXPERIMENTAL

4.1 Synthesis of glycitein

4-Methoxy resorcinol (112) was synthesised according to the previously published procedure. 4-Hydroxy phenyl acetonitrile (113) (9.2 g, 69.1 mmol), 4-methoxy resorcinol (112) (8.0 g, 57.1 mmol) and fused ZnCl₂ (6.1 g, 45.1 mmol) were dissolved into dry ether (240 ml). Dry HCl gas, generated from NaCl, HCl and H₂SO₄, was bubbled into the solution for 5 hours. After bubbling, the violet reaction mixture was left to room temperature over night (14 hours). Ether was decanted off and the gummy solid was washed with dry ether. To hydrolyse the formed ketiminium chloride, 5% HCl (200 ml) was added and the mixture was refluxed for 8 hours. The formed solution was left in the
The yellow crystals were filtered off and washed with water. The recrystallisation from EtOH gave 2,4,4'-trihydroxy-5-methoxy deoxybenzoin (115) (8.1 g, 52%) 1H NMR (200 MHz, acetone-d6): δ = 3.85 (s, 3H, OMe-5), 4.20 (s, 2H, CH2), 6.36 (s, 1H, H-3), 6.80 (d, 2H, J=8.8 Hz, H-3', H-5'), 7.19 (d, 2H, J=8.8 Hz, H-2', H-6'), 7.50 (s, 1H, H-6), 8.25 (s, 1H, OH-4'), 8.89 (s, 1H, OH-4), 12.6 (s, 1H, OH-2).

The cyclisation of (115) was performed as follows. BF3-Et2O (15.8 ml, 124.8 mmol) was added dropwise to a solution of 2,4,4'-trihydroxy-5-methoxy deoxybenzoin (115) (7.0 g, 25.5 mmol) in dry DMF (70 ml) under argon atmosphere. The temperature was raised to 50 °C and a solution of methanesulphonyl chloride (6.1 ml, 79.4 mmol) in dry DMF (20 ml) was added slowly into the reaction mixture. The resulting mixture was then stirred at 100 °C for 2 hours. It was cooled to room temperature and poured into ice water (800 ml) and left in the refrigerator over night. The resulting crystals were filtered off and washed with water. To hydrolyse the partially formylated 7- position the solid was dissolved into 0.5 M NaOH (200 ml) and after stirring for 1 hour at room temperature it was acidified with conc. HCl. The resulting precipitated solid was filtered off and washed with water. Recrystallisation from AcOH gave off white solid (6) (6.5 g, 90%). 1H and 13C NMR (I).

4.2 Synthesis of daidzein-4'-O-β-glucoside

7-O-Bn-daizene (96) (115 mg, 0.33 mmol), Bu4N+Br- (129 mg, 0.40 mmol), K2CO3 (230 mg, 1.67 mmol), CHCl3 (10 ml) and water (10 ml) were placed in a round bottomed flask and the mixture was stirred at room temperature. After obtaining the clear solution (10 min), acetobromo-α-D-glucose (62) (137 mg, 0.33 mmol) was added. After two days another equivalent (137 mg, 0.33 mmol) of acetobromo-α-D-glucose (62) was added. The reaction mixture was stirred for a total of seven days. CHCl3 (40 ml) was added and the separated organic phase was washed with 10% aq. AcOH (2 x 15 ml), water (15 ml), 0.1M Na2S2O3 (15 ml), water (15 ml), sat. NaHCO3 (2 x 15 ml) and water (2 x 15 ml), and dried with MgSO4. The solvent was removed in vacuo to give a solid (326 mg), which was purified by MPLC using CH2Cl2 : MeOH (95 : 5) as an eluent. This gave white solid (244 mg) which was recrystallised from EtOH to give daidzein-7-O-Bn-4'-O-tetraacetylglucoside (148) as white solid (72 mg, 32%) 1H NMR (500 MHz, CDCl3): δ = 2.04-2.09 (4 x s, 12H, Ac), 3.88 (m, 1H, H-2''), 4.18 (d, 1H, J=12.0 Hz, H-6''), 4.30 (dd, 1H, J=13.0 Hz, 5.0 Hz, H-6), 5.11 (m, 1H, H-1''), 5.18 (s, 2H, H-7''), 5.18 (m, 1H, H-3''), 5.30 (m, 1H, H-4''), 5.32 (m, 1H, H-5''), 6.94 (d, 1H, J=2.0 Hz, H-8), 7.06 (d, 2H, J=9.0 Hz, H-3', H-5'), 7.08 (d, 1H, J=2.5 Hz, H-6), 7.37 (m, 1H, H-4''), 7.43 (m, 2H, H-3'', H-5''), 7.46 (d, 2H, J=7.0 Hz, H-2'', H-6''), 7.50 (d, 2H, J=9.0 Hz, H-2'', H-6''), 7.91 (s, 1H, H-2), 8.22 (d, 1H, J=9.0 Hz, H-5), 13C NMR (125 MHz, CDCl3): 20.8 (Ac x 4), 62.1 (C-6''), 68.5 (C-3''), 70.7 (C-4''), 71.3 (C-7''), 71.3 (C-7''), 72.3 (C-2''), 72.9 (C-5''), 99.4 (C-1''), 101.4 (C-8), 115.3 (C-6), 117.2 (C-3', C-5'), 118.7 (C-4a), 124.7 (C-1'), 127.2 (C-3), 127.7 (C-3'', C-5''), 128.0 (C-4''), 128.6 (C-5), 128.9 (C-2'', C-6''), 130.4 (C-2', C-6'), 135.8 (C-1''), 152.4 (C-2), 157.0 (C-4'), 158.0 (C-8a), 163.2 (C-7), 169.5 (C=O), 196.5 (C=O), 170.4 (C=O), 170.7 (C=O), 175.8 (C-4).

Debenzylolation of (148) was performed as follows. A mixture of daidzein-7-O-Bn-4'-O-tetraacetylglucoside (148) (52 mg, 0.08 mmol), thioanisole (0.45 ml, 3.9 mmol) and CF3COOH (2 ml) was stirred at 40 °C for 2 hours. The solvent was evaporated in vacuo
and the product was precipitated from the resulting oil with Et₂O/hexane. The formed solid was filtered off and washed with water. Recrystallisation from EtOH gave daizein-4′-O-tetra-acetylglucoside (149) as white solid (30 mg, 67%). The purity of the sample was verified by LC-MS.

Deacetylation of (149). A suspension of daizein-4′-O-tetra-acetylglucoside (149) (30 mg, 0.05 mmol), KOH (2 mg), EtOH (15 ml) and drop of water, was stirred at room temperature for 5 hours. After completion of the reaction, solvents were evaporated in vacuo, water (10 ml) was added and the solution was neutralised with 10% HCl. The precipitated solid was filtered and washed with water. Recrystallisation from MeOH/H₂O gave daidzein-4′-O-β-D-glucoside (78) as white solid (18 mg, 86%).

1H NMR (500 MHz, DMSO-d₆): δ = 3.18 (m, 1H, H-4´´), 3.27 (m, 1H, H-3´´), 3.29 (m, 1H, H-2´´), 3.35 (m, 1H, H-5´´), 3.48 (m, 1H, H-6´´), 3.70 (m, 1H, H-6´´), 4.55 (s, 1H, OH-6´´), 4.91 (d, 1H, J=6.5 Hz, H-1´´), 5.00 (d, 1H, J=5.0 Hz, OH-4´´), 5.07 (d, 1H, J=3.5 Hz, OH-3´´), 5.31 (d, 1H, J=4.0 Hz, OH-2´´), 6.87 (s, 1H, H-8), 6.94 (d, 1H, J=8.0 Hz, H-6), 7.08 (d, 2H, J=8.5 Hz, H-3´, H-5´), 7.50 (d, 2H, J=7.5 Hz, H-2´, H-6´), 7.97 (d, 1H, J=9.0 Hz, H-5), 8.35 (s, 1H, H-2), 10.79 (s, 1H, OH-7); 13C NMR (125 MHz, DMSO-d₆): 60.7 (C-6´´), 69.7 (C-4´´), 73.2 (C-3´´), 76.6 (C-2´´), 77.0 (C-5´´), 100.4 (C-1´´), 102.1 (C-8), 115.2 (C-6), 116.0 (C-3´, C-5´), 116.6 (C-4a), 123.1 (C-3), 125.5 (C-1´), 127.3 (C-5), 129.9 (C-2´, C-6´), 153.2 (C-2), 157.1 (C-4´), 157.4 (C-8a), 162.5 (C-7), 174.5 (C-4).}

4.3 Regioselective synthesis of isoflavone O-derivatives using t-BuOK as a base

**General synthesis for 4′-O-isoflavone derivatives**

A suspension of isoflavone (300 mg, 1 eq.) and dry t-BuOK (3 eq.) in freshly distilled DMF (40 ml) was stirred at room temperature under Ar for 2 hours. Freshly distilled corresponding halide (1.1 eq.) was added and the reaction mixture was stirred overnight and then poured into water and neutralised with 10% HCl. The precipitated product was filtered off, washed with water, dried in vacuo and purified with MPLC using CH₂Cl₂ : EtOAc (8:2) as an eluent.

The corresponding halides were: AcCl (acetyl protection), BnCl (benzyl protection), BzCl (benzoyl protection) and ethylbromoacetate (ethoxycarbonylmethylglycitein).

Recrystallisation from EtOH gave 4′-O-ethoxycarbonylmethylglycitein (156) as white solid (148 mg, 41%). 1H NMR (500 MHz, DMSO-d₆): δ = 1.21 (t, 3H, J=6.8 Hz, H-4´´), 3.87 (s, 3H, OMe-6), 4.17 (q, 2H, J=7.0Hz, H-3´´), 4.80 (s, 2H, H-1´´), 6.96 (s, 1H, H-8), 6.98 (d, 2H, J=8.5 Hz, H-3´, H-5´), 7.44 (s, 1H, H-5), 7.51 (d, 2H, J=9.0Hz, H-2´, H-6´), 8.34 (s, 1H, H-2), 10.60 (s, 1H, OH-7); 13C NMR (50 MHz, DMSO-d₆): 13.7 (C-4´´), 55.5 (C-OMe), 60.3 (C-3´´), 64.3 (C-1´´), 102.5 (C-8), 104.4 (C-5), 113.8 (C-3´, C-5´), 115.9 (C-4a), 122.2 (C-1´), 124.9 (C-3), 129.7 (C-2´, C-6´), 146.6 (C-6), 151.4 (C-8a), 152.6 (C-2), 152.6 (C-7), 156.9 (C-4´), 168.4 (C-2´´), 173.8 (C-4). HRMS (EI) calculated for: C₂₀H₁₈O₇: 370.1053; found: 370.1068.

Recrystallisation from EtOH gave 4′-O-Ac-daidzein (71) as white solid (150 mg, 43%). 1H NMR (500 MHz, DMSO-d₆): δ = 2.29 (s, 3H, Ac), 6.89 (d, 1H, J=2.5 Hz, H-8), 6.95 (dd, 1H, J=2.0 Hz, 9.0 Hz, H-6), 7.18 (d, 2H, J=8.5 Hz, H-3´, H-5´), 7.60 (d, 2H, J=9.0 Hz, H-2).
Hz, H-2', H-6'). 7.99 (d, 1H, J=9.0 Hz, H-5), 8.42 (s, 1H, H-2), 10.82 (s, 1H, OH-7); $^{13}$C NMR (125 MHz, DMSO-d$_6$): 20.8 (C-Ac), 102.2 (C-8), 115.3 (C-6), 116.6 (C-4a), 121.5 (C-3', C-5'), 121.8 (C-1'), 122.8 (C-3), 127.3 (C-5), 130.0 (C-2', C-6'), 150.1 (C-4'), 153.9 (C-2), 157.4 (C-7), 162.7 (C-5), 169.2 (C=O), 174.3 (C-4).

Recrystallisation from EtOH gave 4'-O-Bn-genistein (142) as white solid (240 mg, 60%). $^1$H NMR (200 MHz, DMSO-d$_6$): $\delta$ = 5.16 (s, 2H, H-7'), 6.24 (d, 1H, J=2.2 Hz, H-6), 6.40 (d, 1H, J=8.8 Hz, H-8), 7.08 (d, 2H, J=8.8 Hz, H-3', H-5'), 7.33-7.52 (m, 7H, H-2', H-6', H-3', H-4', H-5', H-6', H-2', H-6), 8.38 (s, 1H, H-2), 10.92 (s, 1H, OH-7), 12.89 (s, 1H, OH-5); $^{13}$C NMR (50 MHz, DMSO-d$_6$): 69.2 (C-7'), 102.2 (C-8), 115.3 (C-6), 116.6 (C-4a), 121.5 (C-3', C-5'), 121.8 (C-1'), 122.8 (C-3), 127.3 (C-5), 130.0 (C-2', C-6'), 150.1 (C-4'), 153.9 (C-2), 157.4 (C-7), 162.7 (C-5), 169.2 (C=O), 174.3 (C-4).

Recrystallisation from EtOH gave 4'-O-Bn-glycitein (143) as white solid (178 mg, 45%). $^1$H NMR (200 MHz, DMSO-d$_6$): $\delta$ = 3.88 (s, 3H, OMe-6), 5.16 (s, 2H, H-7'), 6.96 (d, 1H, J=8.8 Hz, H-8), 7.07 (d, 2H, J=8.8 Hz, H-3', H-5'), 7.33-7.53 (m, 8H, H-2', H-3', H-4', H-5', H-6', H-2', H-6, H-5), 8.34 (s, 1H, H-2), 10.62 (s, 1H, OH-7); $^{13}$C NMR (50 MHz, DMSO-d$_6$): 55.8 (C-OMe), 69.2 (C-7'), 102.8 (C-8), 114.5 (C-6), 116.2 (C-4a), 122.6 (C-1'), 124.7 (C-3), 127.6 (C-3', C-5'), 127.8 (C-4'), 128.4 (C-2', C-6), 130.1 (C-3', C-5'), 137.1 (C-1'), 147.0 (C-6), 151.7 (C-8a), 152.9 (C-2, C-7), 158.0 (C-4'), 174.2 (C-4).

Recrystallisation from EtOH gave 4'-O-Bz-daidzein (144) as white solid (204 mg, 52%). $^1$H NMR (200 MHz, DMSO-d$_6$): $\delta$ = 3.90 (s, 3H, OMe-6), 6.91 (d, 1H, J=2.2 Hz, H-6), 7.37 (d, 2H, J=8.8 Hz, H-3', H-5'), 7.63 (t, 2H, J=7.0 Hz, H-3', H-5'), 7.68 (d, 2H, J=8.6 Hz, H-2', H-6'), 7.78 (t, 1H, J=7.0 Hz, H-4'), 8.01 (d, 1H, J=8.8 Hz, H-5), 8.17 (d, 2H, J=8.6 Hz, H-2', H-6'), 8.44 (s, 1H, H-2), 10.87 (s, 1H, OH-7); $^{13}$C NMR (50 MHz, DMSO-d$_6$): 69.2 (C-7'), 104.4 (C-8), 114.5 (C-6), 116.6 (C-4a), 121.6 (C-3', C-5'), 122.7 (C-1'), 123.8 (C-3), 127.2 (C-5), 128.9 (C-2', C-6'), 129.8 (C-3', C-5'), 130.0 (C-2', C-6'), 134.0 (C-4'), 136.0 (C-1'), 150.1 (C-4'), 153.9 (C-2), 157.4 (C-8a), 162.6 (C-7), 164.5 (C-7'), 179.8 (C-4).

Recrystallisation from EtOH gave 4'-O-Bz-genistein (145) as white solid (166 mg, 42%). $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ = 6.26 (d, 1H, J=1.5 Hz, H-6), 6.43 (d, 1H, J=2.0 Hz, H-5), 7.38 (d, 2H, J=8.5 Hz, H-3', H-5'), 7.63 (t, 2H, J=8.0 Hz, H-3', H-5'), 7.68 (2H, J=9.0 Hz, H-2', H-6'), 7.77 (t, 1H, J=7.5 Hz, H-4'), 8.16 (d, 2H, J=8.0 Hz, H-2', H-6'), 8.50 (s, 1H, H-2), 10.92 (s, 1H, OH-7), 12.86 (s, 1H, OH-5); $^{13}$C NMR (125 MHz, DMSO-d$_6$): 93.8 (C-8), 99.1 (C-5), 104.4 (C-4a), 121.5 (C-1'), 121.5 (C-3), 121.7 (C-3', C-5'), 124.5 (C-1'), 128.6 (C-3'), 128.9 (C-3', C-5'), 129.8 (C-2', C-6'), 130.1 (C-2', C-6'), 134.0 (C-4'), 150.4 (C-4'), 155.1 (C-2), 157.6 (C-8a), 162.0 (C-5), 164.5 (C-7'), 179.8 (C-4).
3‴, C-5‴), 129.7 (C-2‴, C-6‴), 129.9 (C-2, C-6), 130.0 (C-1‴), 133.9 (C-4‴), 147.0 (C-6), 150.1 (C-4), 151.7 (C-8a), 153.0 (C-7), 153.4 (C-2), 164.5 (C-7‴), 173.9 (C-4).

**General synthesis of 7-O-isoflavone derivatives**

A suspension of isoflavone (300 mg, 1 eq.) and dry i-BuOK (1 eq.) in freshly distilled DMF (40 ml) was stirred at room temperature under Ar for 2 hours. Freshly distilled corresponding halide (1.1 eq.) was added and the reaction mixture was stirred overnight and then poured into water and neutralised with 10% HCl. The precipitated product was filtered off, washed with water, dried in vacuo and purified with MPLC using CH₂Cl₂ : EtOAc (8:2) as an eluent.

The corresponding halides were: AcCl (acetyl protection), BnCl (benzyl protection), BzCl (benzoyl protection), ethylbromoacetate (ethoxycarbonylmethylglycitein) and ethyl-4-bromobutyrate (ethoxycarbonylpropylgenistein)

Recrystallisation from EtOH gave 7-O-ethoxycarbonylmethylglycitein (157) as white solid (242 mg, 67%) ¹H NMR (200 MHz, DMSO-d₆): δ = 1.23 (t, 3H, J=7.0 Hz, H-4‴), 3.90 (s, 3H, OMe-6), 4.20 (q, 2H, J=7.0 Hz, H-3‴), 5.01 (s, 2H, H-1‴), 6.82 (d, 2H, J=8.8 Hz, H-3, H-5‴), 7.20 (s, 1H, H-8), 7.41 (d, 2H, J=8.4 Hz, H-2, H-6‴), 7.47 (s, 1H, H-5), 8.37 (s, 1H, OH-4‴); ¹³C NMR (50 MHz, DMSO-d₆): 13.7 (C-4‴), 55.5 (C-MeO), 60.5 (C-3‴), 65.0 (C-1‴), 101.2 (C-8), 104.3 (C-5), 114.6 (C-3, C-5‴), 117.2 (C-4a), 122.2 (C-1), 122.8 (C-3), 129.7 (C-2, C-6‴), 147.0 (C-6), 150.9 (C-8a), 151.9 (C-2), 152.6 (C-7), 156.8 (C-4‴), 167.5 (C-4). HRMS (EI) calculated for: C₂₀H₁₈O₇: 370.1053; found: 370.1064.

Recrystallisation from EtOH gave 7-O-ethoxycarbonylpropylgenistein (158) as white solid (213 mg, 50%) ¹H NMR (500 MHz, acetone-d₆): δ = 1.23 (t, 3H, J=6.5 Hz, H-6‴), 2.11 (qv, 2H, J=7.0 Hz, H-2‴), 2.52 (t, 2H, J=7.0 Hz, H-3‴′), 4.12 (q, 2H, J=7.5 Hz, H-5‴′), 4.19 (t, 2H, J=6.5 Hz, H-1‴), 6.36 (s, 1H, H-6), 8.48 (s, 1H, OH-4‴), 12.99 (s, 1H, OH-5); ¹³C NMR (125 MHz, acetone-d₆): 14.5 (C-6‴), 25.2 (C-2‴), 31.0 (C-3‴), 60.7 (C-5‴), 68.5 (C-1‴), 93.4 (C-8), 99.2 (C-6), 106.8 (C-4a), 116.0 (C-3, C-5‴), 123.0 (C-3), 124.3 (C-1‴), 131.2 (C-2, C-6‴), 154.6 (C-2), 158.5 (C-8a), 158.9 (C-4), 163.2 (C-5), 165.9 (C-7), 173.2 (C-4‴), 181.8 (C-4).

Recrystallisation from EtOH gave 7-O-Ac-daidzein (141) as white solid (203 mg, 58%) ¹H NMR (500 MHz, DMSO-d₆): δ = 2.33 (s, 3H, Ac-7), 6.83 (d, 2H, J=9.0 Hz, H-3, H-5‴), 7.30 (dd, 1H, J=9.0 Hz, 2.0 Hz, H-6), 7.42 (d, 2H, J=9.0 Hz, H-2, H-6‴), 7.54 (d, 1H, J=2.5 Hz, H-8), 8.16 (d, 1H, J=8.0 Hz, H-5), 8.44 (s, 1H, H-2), 9.56 (s, 1H, OH-4‴); ¹³C NMR (125 MHz, DMSO-d₆): 20.9 (C-2‴), 111.4 (C-8), 115.0 (C-3, C-5‴), 120.0 (C-6), 121.6 (C-1‴), 122.0 (C-4a), 124.0 (C-3), 126.9 (C-5), 130.1 (C-2, C-6‴), 153.8 (C-2), 154.3 (C-7), 156.0 (C-8a), 157.4 (C-4‴), 168.6 (C-1‴), 173.2 (C-4‴), 181.8 (C-4).

Recrystallisation from EtOH gave 7-O-Bn-daidzein (96) as white solid (268 mg, 66%) ¹H NMR (200 MHz, DMSO-d₆): δ = 5.27 (s, 2H, H-7‴), 6.82 (d, 2H, J=8.8 Hz, H-3, H-5‴), 7.15 (dd, 1H, J=2.6 Hz, 9.2 Hz, H-6), 7.25 (d, 1H, J=2.2 Hz, H-8), 7.38-7.52 (m, 7H, H-2‴, H-3‴, H-4‴, H-5‴, H-6‴, H-2, H-6‴), 8.04 (d, 1H, J=9.2 Hz, H-5), 8.37 (s, 1H, H-2), 9.56 (s, 1H, OH-4‴); ¹³C NMR (50 MHz, DMSO-d₆): 70.0 (C-7‴), 101.5 (C-8), 114.9
Recrystallisation from EtOH gave 7-O-Bn-genistein (95) as white solid (256 mg, 64%).
1H NMR (200 MHz, DMSO-d6): $\delta = 5.24$ (s, 2H, H-7”), 6.50 (d, 1H, J=2.2 Hz, H-6), 6.75 (d, 1H, J=2.2 Hz, H-8), 6.83 (d, 2H, J=8.8 Hz, H-3”, H-5”), 7.35-7.50 (m, 7H, H-2”, H-3”, H-4”, H-5”, H-6”, H-2’, H-6’), 8.41 (s, 1H, H-2), 9.62 (s, 1H, OH-4’), 12.92 (s, 1H, OH-5);
13C NMR (50 MHz, DMSO-d6): 69.9 (C-7”), 93.2 (C-8), 98.6 (C-6), 105.4 (C-4a), 127.8 (C-2”, C-6”), 128.1 (C-4”), 126.5 (C-3”, C-5”), 130.1 (C-2’, C-6’), 136.0 (C-1”), 154.4 (C-2), 157.4 (C-4”, C-8a), 161.7 (C-5), 164.1 (C-7), 180.3 (C-4).

Recrystallisation from EtOH gave 7-O-Bn-glycitin (139) as white solid (264 mg, 67%).
1H NMR (200 MHz, DMSO-d6): $\delta = 3.87$ (s, 3H, OMe-6), 5.27 (s, 2H, H-7”), 6.82 (d, 2H, J=8.8 Hz, H-3”, H-5”), 7.33 (s, 1H, H-8), 7.37-7.52 (m, 8H, H-2”, H-3”, H-4”, H-5”, H-6”, H-2’, H-6’), 8.37 (s, 1H, H-2), 9.55 (s, 1H, OH-4’);
13C NMR (50 MHz, DMSO-d6): 55.7 (C-OMe), 70.4 (C-7”), 101.4 (C-8), 104.2 (C-5), 114.9 (C-3”, C-5”), 117.0 (C-4a), 122.5 (C-1’), 123.1 (C-3), 128.1 (C-2”, C-6”), 128.1 (C-4’), 128.5 (C-3”, C-5”), 130.0 (C-2’, C-6’), 135.9 (C-1”), 147.5 (C-6), 151.4 (C-8a), 152.7 (C-2), 153.0 (C-7), 157.1 (C-4’), 174.2 (C-4).

Recrystallisation from EtOH gave 7-O-Bz-daidzein (97) as white solid (257 mg, 61%).
1H NMR (300 MHz, DMSO-d6): $\delta = 6.84$ (d, 2H, J=8.7 Hz, H-3”, H-5”), 7.44 (d, 2H, J=8.4 Hz, H-2”, H-6”), 7.48 (dd, 1H, J=1.8 Hz, 8.7 Hz, H-6), 7.64 (t, 2H, J=8.1 Hz, H-3”, H-5”), 7.76 (d, 1H, J=2.0 Hz, H-8), 7.79 (t, 1H, J=7.2 Hz, H-4’), 8.19 (d, 2H, J=7.2 Hz, H-2”, H-6”), 8.23 (d, 1H, J=9.0 Hz, H-5), 8.48 (s, 1H, H-2), 9.57 (s, 1H, OH-4’);
13C NMR (75 MHz, DMSO-d6): 111.7 (C-8), 115.0 (C-3”, C-5’), 120.1 (C-6), 121.8 (C-1’), 122.0 (C-4a), 124.0 (C-3), 127.0 (C-5), 128.4 (C-1’), 129.0 (C-3”, C-5’), 130.0 (C-2’, C-6’), 130.1 (C-2”, C-6”), 134.4 (C-4’), 153.8 (C-2), 154.5 (C-7), 156.1 (C-8a), 157.4 (C-4’), 164.0 (C-7”), 174.8 (C-4).

Recrystallisation from EtOH gave 7-O-Bz-genistein (98) as white solid (220 mg, 53%).
1H NMR (500 MHz, DMSO-d6): $\delta = 6.85$ (d, 2H, J=8.0 Hz, H-3”, H-5’), 6.87 (d, 1H, J=2.5 Hz, H-6), 7.19 (d, 1H, J=2.0 Hz, H-8), 7.43 (d, 2H, J=9.0 Hz, H-2’, H-6’), 7.63 (t, 2H, J=7.5 Hz, H-3”, H-5’), 7.78 (t, 1H, J=7.5 Hz, H-4’), 8.15 (d, 2H, J=8.0 Hz, H-2”, H-6”), 8.53 (s, 1H, H-2), 9.63 (s, 1H, OH-4’), 13.00 (s, 1H, OH-5);
13C NMR (125 MHz, DMSO-d6): 101.5 (C-8), 105.4 (C-6), 108.9 (C-4a), 115.1 (C-3”, C-5’), 120.8 (C-1’), 123.0 (C-3), 128.4 (C-1’), 129.0 (C-3”, C-5’), 130.0 (C-2’, C-6’), 130.2 (C-2”, C-6”), 134.3 (C-4’), 155.2 (C-2), 155.9 (C-7), 156.4 (C-8a), 157.6 (C-4’), 161.4 (C-5), 163.7 (C-7”), 181.0 (C-4).

Recrystallisation from EtOH gave 7-O-Bz-glycitein (140) as white solid (209 mg, 51%).
1H NMR (500 MHz, DMSO-d6): $\delta = 3.89$ (s, 3H, OMe-6), 6.83 (d, 2H, J=8.0 Hz, H-3”, H-5’), 7.42 (d, 2H, J=8.0 Hz, H-2’, H-6”), 7.63 (t, 2H, J=8.0 Hz, H-3”, H-5’), 7.68 (s, 1H, H-5), 7.78 (t, 1H, J=7.0 Hz, H-4’), 7.80 (s, 1H, H-8), 8.15 (d, 2H, J=8.0 Hz, H-2”, H-6”), 8.45 (s, 1H, H-2), 9.55 (s, 1H, OH-4’);
13C NMR (125 MHz, DMSO-d6): 56.4 (OMe-6), 106.0 (C-5), 113.5 (C-8), 115.0 (C-3”, C-5’), 122.3 (C-4a), 122.4 (C-3), 123.3 (C-1’), 128.0 (C-4’), 129.1 (C-2’, C-6’), 130.0 (C-3”, C-5’), 130.1 (C-2”, C-6”), 134.4 (C-1”).
144.4 (C-7), 149.3 (C-6), 150.0 (C-8a), 153.7 (C-2), 157.3 (C-7´), 163.5 (C-7´´), 174.5 (C-

**Hydrolysis of esters to acids**

Corresponding isoflavone carboxylic acid ester (100 mg), HCOOH (6 ml), water (0.1 ml) and p-toluenesulphonic acid (catalytic amount) were refluxed for 12 hours. After cooling, water (40 ml) was added and the formed suspension was kept at +2°C overnight. Formed crystals were filtered off and washed with water.

Recrystallisation from MeOH/H$_2$O gave 7-O-carboxymethylglycitein (146) as white solid (83 mg, 90%). $^1$H NMR (500 MHz, DMSO-d$_6$): δ = 3.89 (s, 3H, OMe-6), 4.91 (s, 2H, H-1´´), 6.81 (d, 2H, J=8.50 Hz, H-3´, H-5´), 7.15 (s, 1H, H-8), 7.40 (d, 2H, J=8.50 Hz, H-2´, H-6´), 7.47 (s, 1H, H-5), 8.36 (s, 1H, H-2), 9.54 (s, 1H, OH-4´); $^{13}$C NMR (50 MHz, DMSO-d$_6$): 55.5 (C-OMe), 64.8 (C-1´´), 101.0 (C-8), 104.2 (C-5), 114.6 (C-3´, C-5´), 117.0 (C-4a), 122.2 (C-1´), 122.8 (C-3), 129.7 (C-2´, C-6´), 147.0 (C-6), 150.9 (C-8a), 152.1 (C-2), 152.5 (C-7), 169.0 (C-2´´), 173.9 (C-4). HRMS (EI) calculated for: C$_{18}$H$_{14}$O$_7$: 342.0740; found: 342.0749.

Recrystallisation from MeOH/H$_2$O gave 4´-O-carboxymethylglycitein (147) as white solid (84 mg, 91%). $^1$H NMR (500 MHz, DMSO-d$_6$): δ = 3.89 (s, 3H, OMe-6), 4.72 (s, 2H, H-1´´), 6.94 (s, 1H, H-8), 6.95 (d, 2H, J=10.0 Hz, H-3´, H-5´), 7.43 (s, 1H, H-5), 7.49 (d, 2H, J=9.0 Hz, H-2´, H-6´), 8.32 (s, 1H, H-2), 10.60 (s, 1H, OH-7); $^{13}$C NMR (50 MHz, DMSO-d$_6$): 55.7 (C-OMe), 64.3 (C-1´´), 102.8 (C-8), 104.6 (C-5), 114.0 (C-3´, C-5´), 116.1 (C-4a), 122.5 (C-1´), 124.9 (C-3), 129.9 (C-2´, C-6´), 146.9 (C-6), 151.6 (C-8a), 152.8 (C-7), 152.8 (C-2), 157.3 (C-4´), 170.1 (C-2´´), 174.1 (C-4). HRMS (EI) calculated for: C$_{18}$H$_{14}$O$_7$: 342.0740; found: 342.0724.

Recrystallisation from EtOH gave 7-O-carboxylpropylgenistein (159) as white solid (79 mg, 85%). $^1$H NMR (500 MHz, DMSO-d$_6$): δ = 1.96 (qv, 2H, J=7.0 Hz, H-2´´), 2.40 (t, 2H, J=7.5 Hz, H-3´´´), 4.12 (t, 2H, J=6.5 Hz, H-1´´), 6.40 (s, 1H, H-6), 6.65 (s, 1H, H-8), 6.83 (d, 2H, J=8.5 Hz, H-3´, H-5´), 7.39 (d, 2H, J=8.5 Hz, H-2´, H-6´), 8.40 (s, 1H, H-2), 9.59 (s, 1H, OH-4´), 12.16 (s, 1H, OH-4´´), 12.94 (s, 1H, OH-5); $^{13}$C NMR (125 MHz, DMSO-d$_6$): 23.9 (C-2´´), 29.9 (C-3´´´), 67.6 (C-1´´), 92.8 (C-8), 98.4 (C-6), 105.4 (C-4a), 115.1 (C-3´, C-5´), 121.0 (C-3), 122.5 (C-1´), 130.1 (C-2´, C-6´), 154.4 (C-2), 157.5 (C-8a), 161.8 (C-4), 164.4 (C-5), 164.4 (C-7), 174.0 (C-4´´), 180.4 (C-4). HRMS (EI) calculated for: C$_{18}$H$_{14}$O$_7$: 342.0740; found: 342.0724.

4.4 Synthesis of 4´-O-Benzoyl-isoflavones by using K$_2$CO$_3$ as a base.

**General synthesis of 4´-O-Bz-isoflavones**

A suspension of isoflavone (100 mg, 1 eq.) and fused K$_2$CO$_3$ (5 eq.) in freshly distilled DMF (25 ml) were stirred at room temperature under Ar for 2 hours. Freshly distilled BzCl (1 eq.) was added and the reaction mixture was stirred overnight and then poured into water and neutralised with 10% HCl. The precipitated product was filtered off and washed with water, dried in vacuo and purified with MPLC using CH$_2$Cl$_2$: EtOAc (8:2) as an eluent.
Recrystallisation from EtOH gave $4´$-O-Bz-daizein (144) as white solid (69 mg, 49%).

Recrystallisation from EtOH gave $4´$-O-Bz-genistein (102) as white solid (93 mg, 67%).

Recrystallisation from EtOH gave $4´$-O-Bz-glycitein (145) as white solid (46 mg, 35%).

4.5 Synthesis of $7$-O-triacetylglucuronic acid methyl ester $4´$-O-Benzoyl-daidzein

$7$-O-Bz-daizein (97) (100 mg, 0.28 mmol), Bu$_4$N$^+$Br$^-$ (108 mg, 0.34 mmol), K$_2$CO$_3$ (193 mg, 1.40 mmol), CHCl$_3$ (8 ml) and water (8 ml) were placed in a round bottomed flask and the mixture was stirred at room temperature. After obtaining the clear solution (10 min), acetobromo-$\alpha$-D-glucuronic acid methyl ester (70) (111 mg, 0.28 mmol) was added. After two days another equivalent (111 mg, 0.28 mmol) of acetobromo-$\alpha$-D-glucuronic acid methyl ester (70) was added. The reaction mixture was stirred for a total of seven days. CHCl$_3$ (40 ml) was added and the separated organic phase was washed with 10% aq. AcOH (2 x 15 ml), water (15 ml), 0.1M Na$_2$S$_2$O$_3$ (15 ml), water (15 ml), sat. NaHCO$_3$ (2 x 15 ml) and water (2 x 15 ml), and dried with MgSO$_4$. The solvent was removed in vacuo to give a solid (288 mg), which was purified by MPLC using CH$_2$Cl$_2$:MeOH (95:5) as an eluent. This gave white solid (172 mg) which was recrystallised from EtOH to give $4´$-O-Bz-7-O-triacetylglucuronic acid methyl ester daidzein (150) as white solid (64 mg, 34%).

$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 2.06-2.08 (3 x s, 9H, Ac), 3.74 (s, 3H, H-7´´), 4.29 (d, 1H, $J$=9.0 Hz, H-5´´), 5.34 (m, 1H, H-1´´), 5.40 (m, 1H, H-3´´), 5.40 (m, 1H, H-4´´), 7.06 (bs, 1H, H-8), 7.08 (d, 1H, $J$=2.5 Hz, H-8), 7.30 (d, 2H, $J$=8.5 Hz, H-3´, H-5´), 7.52 (t, 2H, $J$=7.5 Hz, H-3´´, H-5´´), 7.63 (d, 2H, $J$=8.5 Hz, H-2´, H-6´), 7.64 (m, 1H, H-4´´´), 8.00 (s, 1H, H-2), 8.22 (d, 2H, $J$=8.0 Hz, H-2´´´, H-6´´´), 8.26 (d, 1H, $J$=8.0 Hz, H-5); $^{13}$C NMR (125 MHz, CDCl$_3$): 20.6 (3 x Ac), 53.1 (C-7´´), 68.9 (C-4´´), 71.0 (C-2´´), 71.6 (C-3´´), 72.8 (C-5´´), 98.4 (C-1´´), 104.5 (C-8), 115.5 (C-6), 120.4 (C-4a), 121.8 (C-3´, C-5´), 124.8 (C-3), 128.3 (C-5), 128.6 (C-3´´, C-5´´), 129.4 (C-1´´), 129.6 (C-4´´), 130.1 (C-2´, C-6´), 130.2 (C-2´´´, C-6´´´), 133.7 (C-4´´´), 151.1 (C-4´), 153.0 (C-2), 157.4 (C-7), 165.1 (C-7´´´), 166.7 (C-6´´´), 169.1 (C=O), 169.3 (C=O), 170.0 (C=O), 175.4 (C-4).  

4.6 Synthesis of biochanin A 7-O-conjugates

4.6.1 $7$-O-ethoxycarbonylpropylbiochanin A

Biochanin A (134) (500 mg, 1.76 mmol), K$_2$CO$_3$ (1.3 g, 8.80 mmol), KI (10 mg) and acetone (50 ml) were placed in a round bottomed flask. Freshly distilled ethyl-4-bromobutyrate (0.38 ml, 2.64 mmol) was added and the mixture was refluxed for 12 hours. After cooling to room temperature the solvent was removed in vacuo, water (100 ml) was added, neutralization with 10% HCl gave a solid which was filtered off, washed with water, dried in vacuo and purified with MPLC using CH$_2$Cl$_2$:EtOAc (8:2) as an eluent. Recrystallisation from EtOH gave $7$-O-ethoxycarbonylpropylbiochanin A (160) as pale yellow crystals (391 mg, 56%) $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 1.19 (t, 3H, $J$=7.0 Hz, H-6´´), 1.99 (qv, 2H, $J$=6.5 Hz, H-2´´), 2.47 (t, 2H, $J$=7.5 Hz, H-3´´), 3.79 (s, 3H, OMe-4´´), 4.09 (m, 2H, H-5´´), 4.12 (m, 2H, H-1´´), 6.40 (s, 1H, H-6), 6.65 (s, 1H, H-8), 7.01 (d, 2H, $J$=8.0 Hz, H-3´, H-5´), 7.52 (d, 2H, $J$=8.5 Hz, H-2´, H-6´), 8.44 (s, 1H, H-
2), 12.91 (s, 1H, OH-5); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 14.1 (C-6´´), 23.9 (C-2´´), 30.0 (C-3´´), 55.2 (OMe-4´), 59.9 (C-5´´), 67.5 (C-1´´), 92.4 (C-8), 98.4 (C-6), 105.4 (C-4a), 113.7 (C-3´, C-5´), 122.2 (C-3), 122.7 (C-1´), 130.1 (C-2´, C-6´), 154.7 (C-2), 157.5 (C-8a), 159.2 (C-4´), 161.7 (C-5), 164.4 (C-7), 172.4 (C-4´´), 180.3 (C-4).

The hydrolysis of 7-O-ethylcarbonylpropylbiochanin A (160) (300 mg) was performed as previously described in procedure 4.3. Recrystallisation from EtOH gave 7-O-carboxypropylbiochanin A (154) as white solid (250 mg, 90%). $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 1.97 (qv, 2H, J=6.5 Hz, H-2´´), 2.40 (t, 2H, J=7.0 Hz, H-3´´), 3.79 (s, 3H, OMe-4´), 4.12 (t, 2H, J=6.5 Hz, H-1´´), 6.41 (d, 1H, J=2.5 Hz, H-6), 6.66 (d, 1H, J=2.5 Hz, H-8), 7.01 (d, 2H, J=9.0 Hz, H-3´, H-5´), 7.52 (d, 2H, J=8.5 Hz, H-2´, H-6´), 8.44 (s, 1H, H-2), 12.91 (s, 1H, OH-5); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 23.8 (C-2´´), 29.9 (C-3´´), 55.1 (C-OMe), 67.5 (C-1´´), 92.8 (C-8), 98.3 (C-6), 105.3 (C-4a), 113.6 (C3´, C-5´), 122.1 (C-3), 122.7 (C-1´), 130.1 (C-2´, C-6´), 154.6 (C-2), 157.4 (C-8a), 159.1 (C-4´), 161.7 (C-5), 164.4 (C-7), 173.9 (C-4´´), 180.2 (C-4).

4.6.2 7-O-(Phthalimide-N-)butylbiochanin A

Biochanin A (134) (500 mg, 1.76 mmol), K$_2$CO$_3$ (1.2 g, 8.79 mmol), NaI (10 mg) were placed in a round bottomed flask. Acetone (40 ml) was added and formed suspension warmed to reflux. N-(4-bromobutyl)phthalimide (546 mg, 1.93 mmol) was dissolved into acetone (10 ml) and added to the refluxing mixture. After the completion of the reaction, the solution was left to cool down and the solvent was evaporated in vacuo, water (100 ml) was added and the solution acidified with 10% HCl. The formed precipitate was filtered, washed with water, dried in vacuo and purified with MPLC using CH$_2$Cl$_2$: EtOAc (8:2) as an eluent. Recrystallisation from acetone gave off white solid (151) (486 mg, 57%). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 1.88 (m, 4H, H-2´´, H-3´´), 3.78 (t, 1H, J=6.5 Hz, H-4´´), 4.06 (t, 1H, J=6.0 Hz, H-1´´), 6.34 (d, 1H, J=2.0 Hz, H-6), 6.37 (d, 1H, J=2.0 Hz, H-8), 6.97 (d, 2H, J=8.5 Hz, H-3´, H-5´), 7.45 (d, 2H, J=9.0 Hz, H-2´, H-6´), 7.11 (m, 2H, H-9´, H-10´), 7.84 (m, 2H, H-8´, H-11´), 7.85 (s, 1H, H-2), 12.81 (s, 1H, OH-5); $^{13}$C NMR (125 MHz, CDCl$_3$): 25.2 (C-3´´), 26.3 (C-2´´), 37.5 (C-4´´), 55.4 (OMe-4´), 67.8 (C-1´´), 92.9 (C-8), 98.6 (C-6), 106.2 (C-4a), 114.1 (C-3´, C-5´), 123.0 (C-1´), 123.3 (C-8´, C-11´), 123.6 (C-3), 130.1 (C-2´, C-6´), 132.1 (C-7´, C-12´), 134.0 (C-9´, C-10´), 152.6 (C-2), 157.9 (C-4´), 159.8 (C-8a), 162.7 (C-5), 164.8 (C-7), 168.4 (C-6´´, C-12´´), 180.8 (C-4).

4.6.3 Pyrazole from 7-O-(Phthalimide-N-)butylbiochanin A

7-O-(Phthalimide-N-)butylbiochanin A (151) (358 mg, 1eq.) and hydrazine hydrate (0.14 ml, 4 eq.) in EtOH (16 ml) were refluxed for 2 hours. After cooling, the solvents were evaporated in vacuo. Recrystallisation from MeOH/Et$_2$O gave white solid (152) (260 mg, 100%). $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ = 1.75 (bs, 4H, H-2´´´, H-3´´´), 2.83 (bs, 2H, H-4´´´), 3.71 (s, 3H, MeO-4´´´), 3.89 (bs, 2H, H-1´´´), 6.08 (3, 2H, H-3´, H-5´), 6.85 (d, 2H, J=6.5 Hz, H-3´, H-5´), 7.29 (d, 2H, J=6.5 Hz, H-2´´´, H-6´), 8.39 (s, 1H, H-5); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 23.9 (C-3´´´), 25.7 (C-2´´´), 38.5 (C-4´´´), 55.0 (OMe-4´´´), 66.6 (C-1´´´), 93.1 (C-3´, C-5´), 95.8 (C-1´), 113.9 (C-3´, C-5´), 121.0 (C-4´), 124.2 (C-
4.7 Synthesis of daunorubicin biochanin A conjugate

Et$_3$N (19 µl, 0.14 mmol) was added to a solution of 7-O-carboxypropylbiochanin A (154) (50 µg, 0.14 mmol) in dry DMF (5 ml) under Ar atmosphere at –16 °C. Pivaloyl chloride (17 µl, 0.14 mmol) was added and the resulting mixture was stirred for 30 min, after which daunorubicin (111) (70 mg, 0.14 mmol) and Et$_3$N (19 µl, 0.14 mmol) in dry DMF (5 ml) were added. The reaction mixture was allowed to warm to room temperature over night (12 h) and poured into water (100 ml). The aqueous layer was extracted with CH$_2$Cl$_2$ (4 x 20 ml), the combined organic layers were washed with 3% NaHCO$_3$ (3 x 20 ml), sat. NaCl (20 ml) and water (2 x 20 ml). The organic phase was dried with MgSO$_4$, filtered and the solvent was evaporated in vacuo. After purification with MPLC using CH$_2$Cl$_2$ : MeOH (95:5) as an eluent, the solid was recrystallised from EtOH/CH$_2$Cl$_2$/hexane to a give bright red solid (153) (45 mg, 38%). $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ =1.14 (d, 3H, $J$=6.5 Hz, H-6´), 1.46 (m, 1H, H-2´), 1.87 (m, 1H, H-2´), 2.09 (m, 1H, H-8), 2.23 (m, 2H, H-3´´´´), 2.67 (s, 3H, H-14), 2.93 (s, 2H, H-10), 3.42 (s, 1H, H-4´), 3.79 (s, 3H, OMe-4´´´), 3.93 (s, 3H, OMe-4'), 4.02 (m, 2H, H-1´´´´), 4.02 (m, 1H, H-3´), 4.19 (m, 2H, H-5´), 4.73 (d, 1H, $J$=5.5 Hz, OH-4), 4.92 (s, 2H, H-7), 5.23 (s, 1H, H-1´), 5.51 (s, 1H, OH-9), 6.31 (s, 1H, H-6´), 6.56 (s, 1H, H-8´), 6.99 (d, 2H, $J$=8.0 Hz, H-3´´´, H-5´´´), 7.48 (d, 2H, $J$=8.5 Hz, H-2´´´, H-6´´), 7.83 (s, 2H, H-1), 8.37 (s, 1H, H-2´), 12.83 (s, 1H, OH-5´´´), 13.24 (s, 1H, OH-11), 13.98 (s, 1H, OH-6); $^{13}$C NMR (125 MHz, DMSO-$d_6$): 17.0 (C-6´), 24.0 (C-14), 24.5 (C-2´´´´), 29.7 (C-2´), 31.4 (C-3´´´´), 31.6 (C-10), 36.2 (C-8), 45.0 (C-3´), 55.1 (C-OMe-4´´´), 56.5 (C-OMe-4), 66.7 (C-5´), 67.9 (C-1´´´´), 68.1 (C-4´), 70.2 (C-7), 75.2 (C-9), 92.7 (C-8´), 98.3 (C-6´), 100.3 (C-1´), 105.3 (C-4a´), 110.5 (C-5a), 110.7 (C-11a), 113.7 (C-3´´´, C-5´´´), 118.9 (C-1), 119.6 (C-3), 119.9 (C-12a), 122.1 (C-3´), 122.7 (C-1´´´), 130.1 (C-2´´´, C-6´´´), 134.4 (C-10a), 134.6 (C-6a), 135.6 (C-4a), 136.1 (C-2), 154.5 (C-2´), 154.5 (C-11), 156.1 (C-6), 157.4 (C-8a), 159.2 (C-4´´´´), 160.7 (C-4), 161.6 (C-5´´´), 164.5 (C-7´), 170.7 (C-4´´´´), 180.2 (C-4´), 186.3 (C-12), 186.4 (C-5), 211.7 (C-13).

4.8 Synthesis of dihydrogenistein

Genistein (5) (500 mg), Pd/C 10% (750 mg) and EtOH (50 ml) were placed in a 2-neck round bottomed flask. The formed suspension was hydrogenated and the reaction was followed by TLC. After the completion of the reaction, the reaction mixture was filtered through a Celite pad and solvent was evaporated in vacuo. The obtained crude product was purified with MPLC using CH$_2$Cl$_2$:EtOAc (8:2) as an eluent. The evaporation of the combined fractions gave dihydrogenistein (21) (342 mg, 68%). $^1$H and $^{13}$C NMR (I)

5. CONCLUSIONS

This thesis combines the available literature on the synthesis of isoflavones and their conjugates, including sulphates, glycosides, protecting groups, various alkyl chains and carboxylic acids, as well as deuteration and chlorination. This information is valuable
when planning synthetic routes towards new possible metabolites for biological screening either originated from mammals or from bacterial broths. The available literature is divided in chapters according to functionalities and discussed in details. Traditional synthetic approaches for phenolic derivatives are also included, since the synthesis of isoflavone O-conjugates arises from those grounds.

The results of this study have provided us with a number of new ways to synthesise isoflavone derivatives. These include the di-O-sulphates of three major isoflavones, daidzein (4), genistein (5) and glycitein (6) and di-O-sulphates of their reduced forms, dihydrodaidzein (20), dihydrogenistein (21) and equol (19). A new efficient microwave assisted synthetic method for obtaining deuterated isoflavones was developed. Using CF₃COOD under microwave irradiation, daizein-d₆ (88), genistein-d₄ (122) and glycitein-d₆ (123) were obtained in high yield and isotopic purity. With the above mentioned sulphation method, the di-O-sulphates of these deuterated isoflavones were obtained in moderate yield (124, 125, 126). This method is a useful tool when isotopically labelled isoflavones and their metabolites are required.

One of the most challenging tasks was the synthesis of daidzein-7-O-β-D-glucuronide-4′-O-sulphate (16). Since the compound is highly polar and sensitive, the reaction steps had to be optimised carefully. This synthesised compound can be used in the development of analytical methods to further understand its biological significance and activity as well as the metabolic circulation of this particular compound in human.

Different protecting groups were investigated during this study. Optimised protection procedures for introducing Bn and Bz groups selectively at the position 7-O and 4′-O by direct introduction or by transacylation can be used in the further study of the synthesis of naturally occurring isoflavones and their metabolites. The use of a non-hydrogen assisted method (push and pull) for debenzylation discloses the risk of reduction of the double bond at C-ring. Still, pressure-controlled hydrogenation reactions for debenzylation should be further studied.

The aromatic chlorination method using neat thionyl chloride was a result of the unexpected chlorination during synthesis of isoflavone acid chloride. This developed method can be used to produce 8-chlorogenistein (131), 6,8-dichlorogenistein (108) and 6,8-dichlorobiochanin A (136). The suitability of this chlorination method should be investigated for other bacterially produced chlorinated compounds owning the corresponding hydrogen bonded OH group at para position. The results of the chlorination of genistein (5) and biochanin A (134) with thionyl chloride can be used in the bromination studies of these compounds with thionyl bromide.

Daidzein-4′-O-β-glucoside (78) was synthesised using protecting group chemistry. This same method can be used in the synthesis of genistein-4′-O-β-glucoside (77). In connection with, transacylation was noted to be a useful tool in the synthesis of daidzein-7-O-triacetylglucuronide-4′-O-Bz (150), starting from daidzein-7-O-Bz (97). The usefulness of transacylation should be considered also with other ester bonded isoflavone derivatives.

The use of a Boc₂NR moiety instead of the phthalimide group for producing alkylamine chain derivatives of isoflavones should be studied.²²² Boc protection can easily be cleaved
under acidic conditions without any fear of decomposition of the isoflavone skeleton. The
use of hydrazine hydrate for the cleavage of phthalimide group resulted in a reaction at C-
ring forming a pyrazole. This result can be used for producing pyrazoles also from
daidzein (4), genistein (5), glycine (6) and biochanin A (134) and study the possible
biological activity of these compounds.

The biochanin A daunorubicin conjugate (153) was synthesised and characterised. The
biological activity was tested on MCF7 and Ishikawa cell lines, showing negligible
activity. However, other potential cell lines will be tested in the future. The synthetic
method described here can be exploited for other isoflavones and isoflavonoids to test
their possible activity. One interesting compound of these could be equol (19) which has
shown biological activity in human.

As we have now seen, new synthetic approaches towards isoflavone O-conjugates were
developed during this study. These new methods will hopefully take the synthetic research
of isoflavones and their metabolites one step further. It remains to be seen if these
synthesised compounds will be useful in the development of analytical methods and in
biological studies.
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