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**SYNTHESIS AND CHARACTERISATION OF  
BIOMATERIALS FOR USE AS MARKERS OF  
HEALTH AND DISEASE**

A thesis presented in partial fulfilment of the requirements for the degree of

**Doctor of Philosophy**

in

**Chemistry**

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**Amended, 2001**

## ABSTRACT

The bicyclic[4.3.0]nonane ring system is commonly found in many complex bioactive natural products, such as spongioids and several novel steroids. Previous examples of ester tethered cycloaddition reactions were limited to activated dienes and dienophiles. The synthesis of a range of precursors, in which an unactivated diene and dienophile were linked *via* an ester tether is described. Model studies into the synthesis of monocyclic and bicyclic lactones as possible pre requisites for the formation of the C/D rings of the spongioid skeleton utilising intramolecular Diels-Alder reaction (IMDA) or alternative cyclisation methods such as free radical catalysed and Heck reaction were carried out with these substrates. However it was discovered that when the carbonyl group of the ester tether was in conjugation with the diene it caused a formidable challenge as none of the applied methods were found to be suitable for the cyclisation reactions.

Chapters two to five were focused on attempts to synthesise glucuronides of phytoestrogen metabolites (isoflavones and isoflavans) glucuronide due to their potential interest as anti cancer agents. Also the steroidal hormone estrone glucuronide (for fertility testing) and testosterone glucuronide (for use in clinical laboratories and for drug testing) for the purpose of developing multipurpose home monitor by adapting the platform technology previously developed and used in a point-of-care monitoring device known as the Ovarian Monitor.

The synthesis of phytoestrogen glucuronide is a relatively new concept and the literature revealed no successful chemical method to date. The desired phytoestrogen isoflavones required for stereoselective glucuronidation were successfully prepared from precursor deoxybenzoins using a new convenient and facile route. Reduction of the isoflavones to isoflavans was also carried out using standard literature procedures. Various activated and deactivated phenols (including a sterically hindered phenol) were successfully glucuronidated using various synthetic routes as model studies. The information garnered from the model studies was utilised for the glycosylation of isoflavones and isoflavan but numerous attempts to obtain the glucuronides by using direct methods failed. Even more reactive glycosyl donors such as the sulfoxide sugar and acetimidate sugar also failed to effect glycosylation of these phytoestrogen metabolites. The relative insolubility and instability of the chromene ring under acid-base reaction conditions were compounding problems for the isoflavones.

A new alternative route involving synthesis of the *O*-glucuronides by the prior synthesis of the glycosides, hydrolysis to the glucosides and then TEMPO mediated selective oxidation of the primary alcohol was successful for simple phenol, sterically hindered phenol and the steroids estrone and testosterone. However, this alternative route also failed to effect glucuronidation of the isoflavones. Attention was thus focused on the synthesis of isoflavone glucuronides using UDP-glucuronyl transferase. Towards this end ( $\pm$ ) methoxy equol glucuronide has been synthesised enzymatically, and purified using chromatographic methods. The attempted enzymatic synthesis of formononetin gave instead the cleavage product 2-hydroxy, 4'-methoxy deoxybenzoin glucuronide. The glucuronides were fully characterised by  $^1\text{H}$ - $^1\text{H}$  2D-COSY,  $^1\text{H}$ - $^{13}\text{C}$  2D-HETCOR and DEPT spectra and the results unambiguously showed the  $\beta$ -linkage of the glucuronide ring with the aglycon moieties. The presence of the glucuronide ring at the C-4 position in 2-hydroxy, 4'-methoxy deoxybenzoin glucuronide was also confirmed by a long range coupling experiment (HMBC).

The stereochemical integrity of the estrone glucuronide (EIG) obtained using perester coupling, acetimidate coupling and TEMPO catalysed oxidation methods were clinically tested by comparison with a standard curve obtained with a sample produced by the Koenigs-Knorr method. Testosterone glucuronide was studied for use as a biomarker to validate the concept of a multi-purpose home monitor for a variety of analyte glucuronides of clinical interest. Testosterone glucuronide antibodies with high affinity were generated by immunisation of sheep and testosterone glucuronide-HEW lysozymes conjugates were prepared. The standard curve for TG clearly showed it can be used for measurement of urinary TG at physiological concentrations. This methodology can be extended for analyte glucuronides of interest and can be used for development of biomarkers for health and disease. Thus a multi-purpose home monitor is now a reality and exciting commercial and practical applications are expected in the future.

## Errata

Page	Line	Amendment
ii	18	an attempts should read attempts
iii	2	<u>obtain</u> the glucuronides <u>by</u>
vi	21	<u>tetraene</u>
viii	28	isofl <u>avone</u>
ix	1	<u>donors</u>
xiv	15	spectroscopy
xv	5	fourier
23	Scheme 1.16	hydrogenation
82	-	Figure 1.1 <u>8</u>
95	11	thyroglobulin
96	-	<u>Ag</u> -Ab complex
108	9	al <u>l</u>
114	-	2.15, 2.16, 2.18, 2.19 should read R <sub>2</sub> =H
116	17	posi <u>tion</u>
120	29	Black <u>well</u>
123	5	<u>led</u>
126	6	stereochemi <u>stry</u>
129	5	Cyclopentadienyl
130	7	separ <u>ation</u>
131	14	<u>boron</u>
141	7	pyrr <u>olidine</u>
142	1	<u>re-esterification</u>
142	4	phosphor <u>us</u>
143	7	selec <u>tivity</u>
145	8	intermediate
145	11	phosphor <u>us</u>
145	16	benzyl
152	13	recrystall <u>ised</u>
156	4	Tri <u>hydroxy</u>
171	13	Friedel-Cra <u>fts</u>
178	14	ac <u>etic</u>
181	8	recrystall <u>isation</u>
182	14	m-prep <u>thalic</u>

Page	Line	Amendment
183	8	K <u>Mn</u> O <sub>4</sub>
184	15	convenient <u>ly</u>
186	30	( should be <u>g</u>
187	4	<u>Yield</u>
189	-	OTs should be OH (3.124)
190	2	<u>more</u> deactivated
193	7	cleavag <u>e</u>
193	5	bromosug <u>ar</u>
194	3	glucuronidat <u>ion</u>
202	7, 9	ac <u>etyl</u>
205	5	sulf <u>oxide</u>
209	24	<u>Lett.</u>
211	12	1988, 42 <u>B</u>
212	1	United <u>s</u> tates
219	19	<u>4.2</u>
247	4	<u>Wellington</u>
251	4	ac <u>etyl</u>
264	11	2,6-dimethyl <u>phenol</u>
266	2	<u>S</u> tevenson
267	7	chromen <u>e</u>
271	3	glucur <u>onide</u>
282	21	<u>regioselectivity</u>
288	21	desicc <u>ation</u>
291	26	test <u>osterone</u>
292	2	dichlor <u>ophosphate</u>
306	13	recrystall <u>isation</u>
318	5	Bowers <sup>15</sup>
318	23	co-workers <sup>18</sup>
337	31	<u>Lett.</u>
338	5	ster <u>oid</u> .

All chemical names with propionate should read propynyl ester in Chapter 1.

Scheme numbers should move up by text 1 in pages 61-77. 1.30 should read 1.31, 1.31 should read 1.32 and so on.

**This thesis is dedicated to my parents :**

**Natverlal K. Desai and**

**Manjula N. Desai**

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**ABBREVIATIONS**

$\Delta$	reflux
$A_{280}$	absorbance at 280 nm
AB	antibody
Ac	acetyl
AcOH	acetic acid
AIBN	2, 2'-azo- <i>bis</i> -isobutyronitrile
Aq.	Aqueous
Ar	aryl
BHT	2,6-di- <i>tert</i> -butyl-4-methylphenol
Bn	benzyl
BSA	bovine serum albumin
BTEAB	benzyl triethyl ammonium bromide
Bz	benzoyl
COSY	correlated spectroscopy
$\text{CH}_2\text{Cl}_2$	dichloromethane
d	day(s) or doublet
DA	Diels-Alder
DCC	dicyclohexylcarbodiimide
DEPT	distortionless enhancement by polarization transfer
DIBAL-H	diisobutylaluminium hydride
DMAP	<i>N,N</i> -dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethylsulfoxide
E1G	estrone glucuronide
E1G[H]	estrone glucuronide (acid form)
EI	electron impact
EIA	enzyme immunoassay
eq	molar equivalents
$\text{Et}_2\text{O}$	diethyl ether
EtOAc	ethylacetate
EtOH	ethanol

Et <sub>3</sub> N	triethylamine
eV	electron Volts
FMO	frontier molecular orbital
FPLC	Fast protein liquid chromatography
FT	Fourier transform
h	hour(s)
H <sub>2</sub> O	water
Hex	hexane
HETCOR	heteronuclear chemical-shift correlation spectroscopy
HEWL	hen egg white lysozyme
HMBC	heteronuclear multiple bond correlation spectroscopy
HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
Hz	hertz
I.D.	internal diameter
IR	infra-red
IMDA	intramolecular Diels-Alder reaction
LUMO	lowest unoccupied molecular orbital
M	mol L <sup>-1</sup>
MA	maleic anhydride
Me	methyl
MeOH	methanol
Min	minute
MP	melting point
NHS	<i>N</i> -hydroxysuccinimide
NK	natural killer
NMR	nuclear magnetic resonance
Ph	phenyl
PhMe	toluene
PhH	benzene
ppm	parts per million
Py	pyridine
RIA	radio immunoassay
R <sub>f</sub>	retention factor

RT	room temperature
s	singlet
S.M.	starting material
SOMO	singly occupied molecular orbital
t	time or triplet
$\Delta T$	change in transmission
TBHS	tetrabutyl ammonium hydrogen sulfate
TBAB	tetrabutyl ammonium bromide
TG	testosterone glucuronide
TIMC	tandem intramolecular cyclisation
TIMDA	tandem intramolecular Diels-Alder
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)aminomethane
WHO	world health organisation