Contemporary Medicinal Chemistry of Glucuronides

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Topics

- The glucuronidation process: common drug examples
- Preventing glucuronidation
- Some glucuronide syntheses, with practical details:
  - Less usual structural types; imidate glucuronidation;
  - the elimination reaction
- Enzymatic glucuronidation including recombinant UGTs
- Acyl glucuronides: Synthesis, structure-reactivity
- In vivo protein adducts of diclofenac acyl glucuronide
**Glucuronidation** is a fundamental process in Phase II metabolism, whereby a wide range of functional groups—including those generated as primary metabolites—may be converted into highly water-soluble, readily excreted glucuronides.

- The cofactor UDPGA is widely distributed in the body—especially the liver
- *Fifteen* UGT isoforms have been identified as responsible for human glucuronidation
- Alcohols, phenols, carboxylic acids and amines can all be derivatised this way
- The primary product is always the 1β-glucuronide, as shown
- Some glucuronides, notably *N*-glucuronides, are not detected in pre-clinical toxicology
- A few glucuronides of well-known drugs…
Morphine-6-glucuronide is a well-known example of a pharmacologically active glucuronide; the major *in vivo* metabolite of morphine is the (inactive) 3-glucuronide (M3G: M6G ~ 5:1).
Preventing glucuronidation

*Two case studies*

- The anaesthetic propofol is significantly metabolised to its *O*-glucuronide.
- This metabolism was very largely associated with the UGT1A9 isoform.
- Addition of a closely related phenol, 2,5-isopropyl phenol, led to sacrificial glucuronidation of the additive:

\[
\text{Propofol} \rightarrow \text{2,5-DIP}
\]

Recombinant human enzyme (in insect cell microsomes) was used:

*Drug Metab. Lett.* 2007, 1, 77-79.
A series of carboxylic acid derivatives was evaluated as 11β-HSD1 inhibitors. Acyl glucuronidation was a major clearance pathway for, e. g. the early lead compound AZD4017:

![AZD4017](image)

A detailed SAR study showed that both bicyclic and aryl carboxylic acid analogues of this series (combined with a heterocycle switch for the latter) led to greatly reduced acyl glucuronidation…

![AZD8329](image)

Lundbeck’s antidepressant Lu AA21004 was metabolised, via the hydroxylamine, to a mixture of \( N-O \) and *quaternary ammonium* glucuronides:

\[ \text{M1 was obtained in sufficient amounts by incubation with human liver microsomes; M2 required chemical synthesis…} \]
The carbohydrate intermediate was available from the familiar bromosugar…
A modified Konigs-Knorr synthesis, with 2-phase alkylation, was very effective: brief deprotection selectively removed the phthalimide.

*Drug Metab. Dispos.* 2011, **39**, 2264-2274.
A carbamoyl glucuronide example

As well as direct $N$-glucuronidation, amines can also metabolise as carbamoyl glucuronides. E. g. the antiarrhythmic agent, mexiletine:

- Glucuronidation was highly enantioselective $\Rightarrow$ (R)-glucuronide
- Synthesis began with the (R)-carboxylic acid
- Unlike $O$-acyl glucuronides, carbamoyl glucuronides are stable to brief base hydrolysis

**Imidate glucuronidation- often superior to Konigs-Knorr**

For alkyl glucuronidation, the Schmidt imidate is often invaluable, e.g. **17α-boldenone**:

Here the Konigs-Knorr reaction failed- though it was fully satisfactory for the 17β-OH epimer. *Steroids* 2009, 74, 250-255.

Further example- human metabolite of the anticancer trial compound ABT-751:

Semisynthetic artemisinins such as **artemether** metabolise via CYP-mediated dealkylation, then glucuronidation. The human glucuronide proved to have 12α, 1´β- stereochemistry.

Conformational analysis of ring D of dihydroartemisinin:

Using *acetate* rather than *isobutyrate* protection, yield was <10%.

Beware *elimination* from glucuronides!—either in the final hydrolysis or whenever a strong base is used.

HATU, NMM, cat. DMAP was even better (74%, no dehydro product) *J. Med. Chem.* 2011, **54**, 4119-4132.
Enzymatic Glucuronide Synthesis

- Hepatic microsomes, recombinant human UGTs and engineered glycosynthases all offer possible routes—and may be regioselective in some cases.
- **Example-species-selective glucuronidation of an oral antithrombotic candidate:**

Cf. human liver microsomes: almost entirely the 3-glucuronide.

*Drug Metab. Dispos.* 2006, **34**, 1502-1507.
Human UGTs- site selectivity:

Mycophenolic acid- an important immunosuppressant, e. g. in transplantation:

…here again, the acyl glucuronide retains some on-target activity.

*Drug Metab. Dispos.* 2005, **33**, 139-146.

**Use of an engineered glycosynthase**

- Wild type glucuronidase ex *E. coli* (EC 3.2.1.31)
- Key mutation, Glu504 → Gly/Ala/ Ser, abolishes hydrolytic activity
- A glucuronyl fluoride was used as the glycosyl donor
- A range of *O*-alkyl glucuronides made, generally good yields
Acyl Glucuronides

O-Acyl (ester) glucuronides are undoubtedly protein reactive.  Are they toxic metabolites?

Many well-known drugs, including NSAIDS, are significantly metabolised as acyl glucuronides—see below.

- Considering first chemical reactivity, the nature of R is important:
  - R = aryl: Reactivity predictable using Hammett considerations
  - R = alkyl: Degree of α-substitution is very important...
    - R = CH₂R’, R = CHMeR’, R = CMe₂R’ show a clear gradation of properties.

- Ibuprofen
- Diclofenac
- Mycophenolic Acid
Chemical Reactivity of Acyl Glucuronides

In addition to direct reaction with nucleophiles (hydrolysis or amination by e. g. lysine residues in proteins), acyl glucuronides may undergo acyl migration in a base-catalysed process...

- Acyl migration is rapid in vitro (e. g. aq. buffer) at pH 6.5 or greater; $t_{1/2}$ increases with the degree of $\alpha$-substitution, e. g. Me$_2$C> MeCH> CH$_2$.
- Transacetylation (by external nucleophiles) is significantly slower for 2/3/4-O-acyl isomers.
- The acyl migrated species may also react with nucleophiles, e. g. Lys-NH$_2$, by addition at C(1) followed by rearrangement (glycation-Amadori pathway).
- *Only* 1$\beta$-acyl glucuronides are good substrates for glucuronidases.
Using minimal carbohydrate protection and highly selective acylation, a wide range of 1β-O-acyl glucuronides can be prepared with excellent stereoselectivity...

Allyl and PMB esters are also useful; compatibility of functionality in R will decide which is appropriate.

In Vitro Stability of O-Acyl Glucuronides

- NMR studies in buffer at pH 7.4 show a clear structure-reactivity profile.
- For the profens and related NSAIDs, \( \alpha \)-substitution is key.
- Composite reaction is a mixture of transacylation and hydrolysis:

When \( X = H \):

<table>
<thead>
<tr>
<th>Acyl glucuronide</th>
<th>( k_t ) h(^{-1} )</th>
<th>( t_{1/2} ) h</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_1 = R_2 = H )</td>
<td>2.353</td>
<td>0.29</td>
</tr>
<tr>
<td>( R_1 = \text{Me}, R_2 = H ) (2R)-</td>
<td>0.903</td>
<td>0.78</td>
</tr>
<tr>
<td>( R_1 = H, R_2 = \text{Me} ) (2S)-</td>
<td>0.405</td>
<td>1.71</td>
</tr>
<tr>
<td>( R_1 = R_2 = \text{Me} )</td>
<td>0.029</td>
<td>23.30</td>
</tr>
</tbody>
</table>

In this series, \( k_h < 10\% \) of \( k_t \).

To a good approximation, the first-order NMR degradation rate reflects \( k_t \).

Transition state analysis for the transacylation step gave excellent correlation with NMR data. Especially, for the monomethyl compounds, $k_d (2R) \sim 2x k_d (2S)$:

\[ ...the (2S)- isomer has to adopt the higher energy TS2. Empirically the ‘twofold rule’ was well known. \]
The twofold R/S difference still applies: $t_{1/2}$s are \textbf{1.8h, 3.7h} for R/S Ibu

The remote isobutyl group has a substantial effect

Esters (R= ethyl, allyl) also greatly slow the degradation:

\[ t_{1/2} = 7.24h \text{ (ethyl), 9.24h (allyl)} \]

For two significant \textit{in vivo} metabolites (R = H):

\[ R_1 = \text{OH}, R_2 = \text{H}, t_{1/2} = 5.03h; \ R_1 = \text{H}, R_2 = \text{CO}_2\text{H}, t_{1/2} = 4.80h \]

Also the ionised carboxylate plays a part-different SAR for corresponding glucosides

At present it is difficult to rationalise these long-range effects

**O-Acyl Glucuronides in Plasma**

Considering the ibuprofen-related series, in human plasma @ 37°C:

- R₁ = R₂ = H, Ibufenac
- R₁ = R₂ = H/Me, (R/S)-Ibuprofen
- R₁ = R₂ = Me, ‘Bibufenac’

- **In plasma**, rates of reaction are *higher* for all compounds compared to buffer
- Hydrolysis now greatly predominates over transacylation
- At a concentration of 2 µg/mL, t₁/₂s in **plasma** are:
  - Ibufenac 0.27 h
  - (R)-Ibuprofen 0.36 h
  - (S)-Ibuprofen 0.22 h
  - Bibufenac 5.2 h (2 µg/mL)

…from 5 to 18-fold lower.

- **HPLC-MS measurement necessary as NMR now impractical**

_Xenobiotica_ 2010, **40**, 9-23.
Case Study- An unusually reactive O-acyl glucuronide

A candidate from a series of neutral endopeptidase inhibitors by Pfizer was withdrawn on toxicological grounds. Here the acyl glucuronide had a very short half life,…

Culprit is the NH: pKₐ ~ 9.5.

Cf. another member of the series…

Here, pKₐ (NH) ~ 16: the acyl glucuronide has t₁/₂ ~50 h.
Preparation of the acyl glucuronide required NH protection:

- The benzyloxymethyl (Bom) group has been used in peptide synthesis
- Other N-protection (allyl, Boc, Z) not satisfactory
- The final AG is highly stable at pH ~ 3; rapidly cyclises at < physiological pH
The cyclic compound (glutarimide) was easily made independently:

...as expected, it reacts readily with nucleophiles:
- The Lys adducts were stable at pH 7.4
- The Cys adducts rapidly degraded at pH 7.4 but were stable at pH 3.0 for several hours.

The acyl glucuronide showed similar reactivity to the imide with nucleophiles including hydrolysis
- …we concluded that intermediacy of the imide explained the reactivity of the AG

*J. Med. Chem. 2007, 50, 6165-6176*
Proteomics of Diclofenac Acyl Glucuronide

- Diclofenac AG has a short half-life and is known to be protein reactive in vitro.
- We sought protein adducts of the AG in diclofenac patients without adverse drug reactions.
- Other important diclofenac metabolites are the 4′- and 5-hydroxy derivatives:

...both are oxidised to quinoneimines which can deplete glutathione.

Diclofenac quinone imine forms albumin adducts in vitro

NB: the 5-OH metabolite is the more readily oxidised; the derived QI is shown here.
Synthesis of diclofenac AG:

...we obtained a batch of 550 mgs, pure 1β-anomer, for the in vitro studies.
**In vitro** protein reactivity:

- Diclofenac AG was incubated with human serum albumin (HSA) at 50:1 molar ratio
- Of 59 Lys residues in HSA, *eight* were consistently modified
- Both acylation and glycation were observed:

  - Lys *acylated* adducts arise principally from the starting 1β-AG isomer
  - Apparently the 2/3/4-O-acyl isomers all contribute to *glycation*
  - In human plasma, *hydrolysis* dominates - cf. ibuprofen
In vivo protein reactivity: Clinical plasma samples

- Six patients took part in the study
- They had taken diclofenac @ 100-150 mg/ day for at least 1 yr.
- 1-3 h after the last tablet, single plasma samples were taken and acid-stabilised
- A total of seven adducted residues and ten modified peptides were identified after tryptic digest of HSA
- The most common modification was transacylation but glycation was also seen
- In one case, glucuronylation (slow, direct reaction of HSA with glucuronic acid) was observed
- We conclude that HSA adduction is not, invariably, a causation of adverse drug reactions with diclofenac
- ...and diclofenac AG is not directly cytotoxic in hepatocytes or kidney cells


Structures...
Diclofenac forms albumin glycation adducts in man

Glycation adduct

\[ \text{DCF-AG-Lys199 adducts detected in man} \]

\[ ^{198}\text{LK(DCF-AG)C(iodo)ASLQK}^{205} \]
Diclofenac forms albumin *acylation* adducts in man

**Acylation adduct**

\[
\text{DCF-AG-Lys199 adducts detected in man}
\]
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