UNIVERSITY OF LIVERPOOL

Contemporary Medicinal Chemistry of Glucuronides

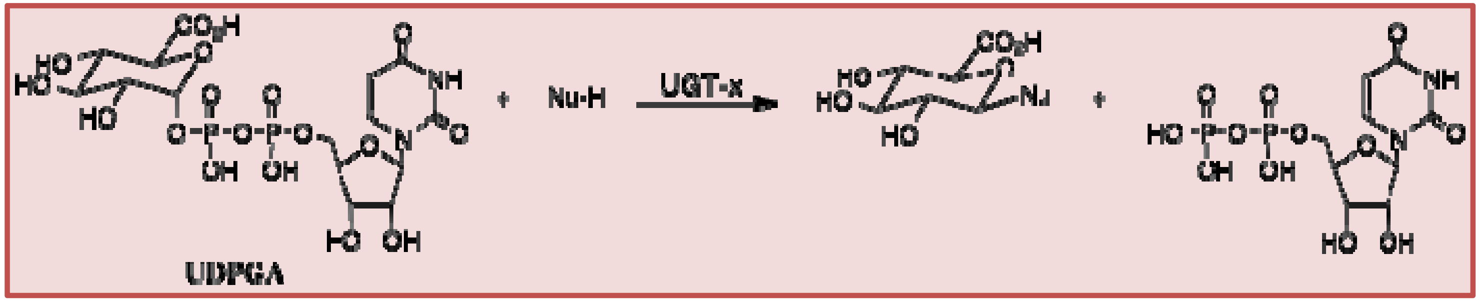
Andrew Stachulski, Feb. 10th 2014

DMPK Meeting, RSC, London

Topics

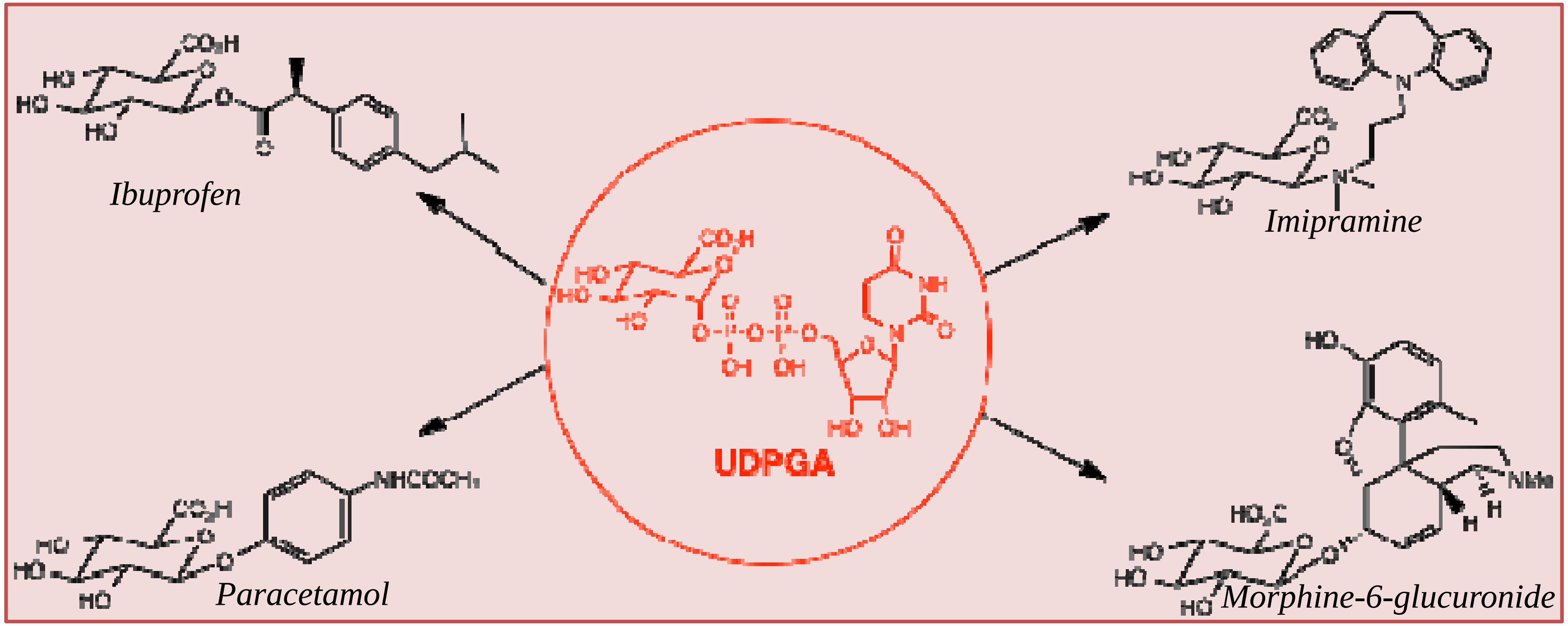
- The glucuronidation process: common drug examples
- Preventing glucuronidation
- Some glucuronide syntheses, with practical details:
  Less usual structural types; imidate glucuronidation;
  the elimination reaction
- Enzymatic glucuronidation including recombinant UGTs
- Acyl glucuronides: Synthesis, 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-maybe converted into highly water-soluble, readily excreted glucuronides.



Nat. Prod. Rep. 2013, **30**, 806-848.

- 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)

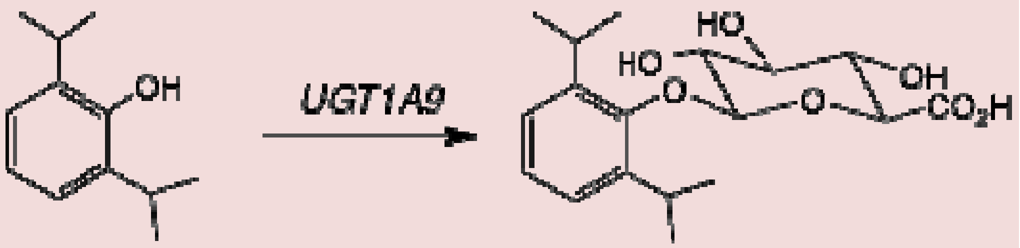
Preventing glucuronidation

Two case studies

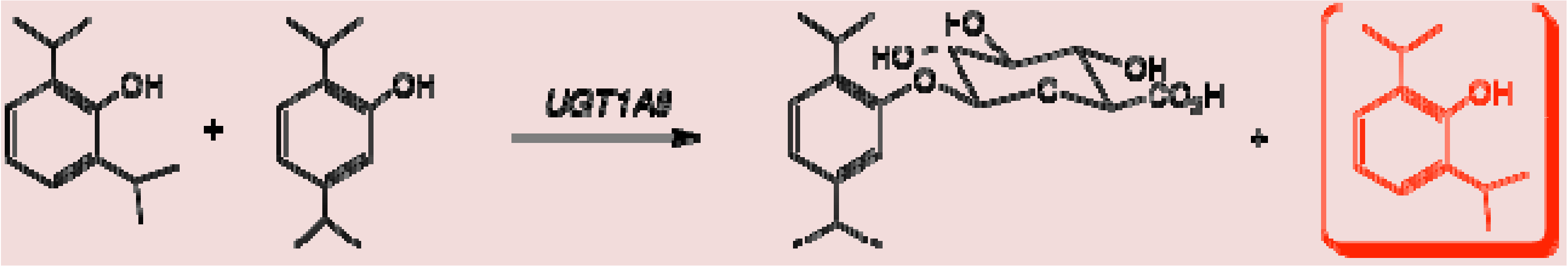
•The anaesthetic propofol is significantly metabolised to its *O*-glucuronide

•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:



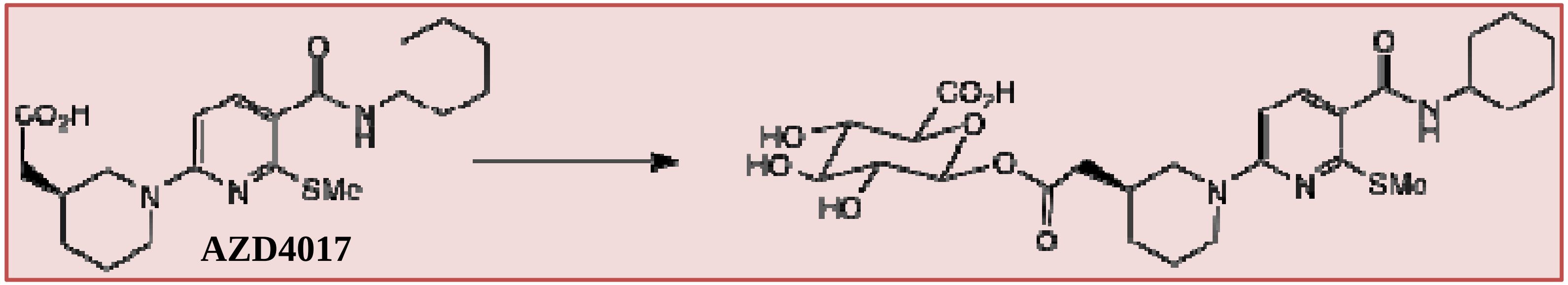
Propofol **2,5-DIP**



Recombinant human enzyme (in insect cell microsomes) was used: *Drug Metab. Lett.* 2007, **1**, 77-79.

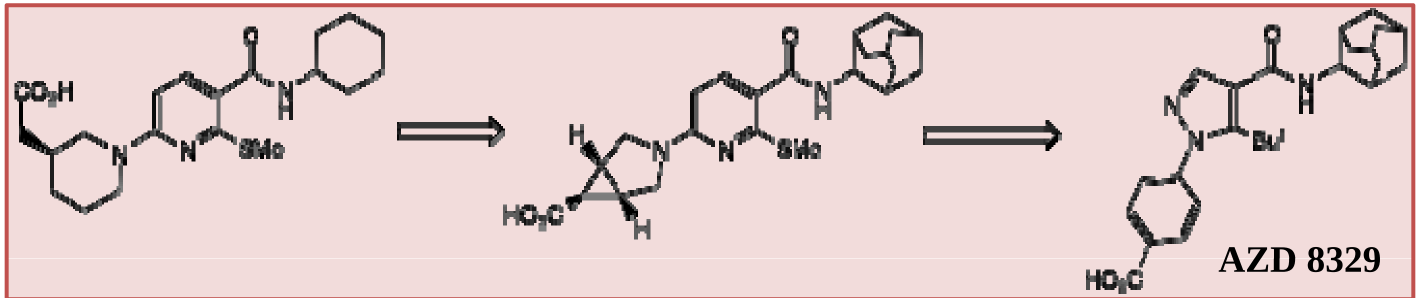
Preventing glucuronidation
-Carboxylic acid drug examples

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

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…



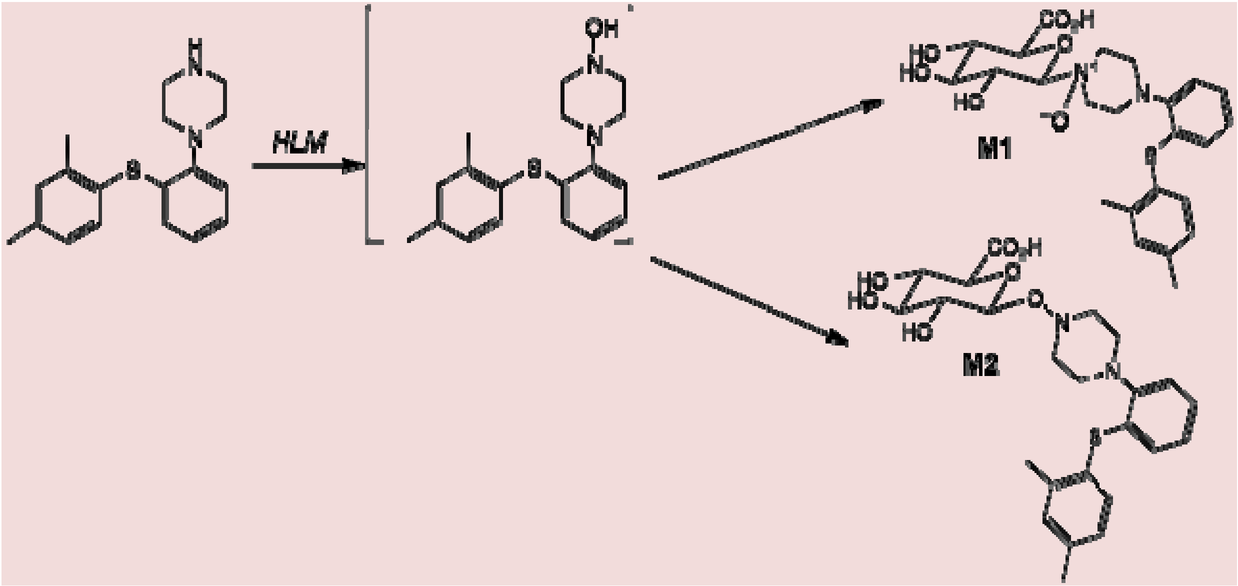
AZD 8329

…leading to **AZD 8329**. As well as the electronic/ steric character of the $–CO_2H$, overall lipophilicity was very important. *Med. Chem. Comm.* 2012, **3**, 1264-1269; *J. Med. Chem.* 2012, **55**, 10136-10147.

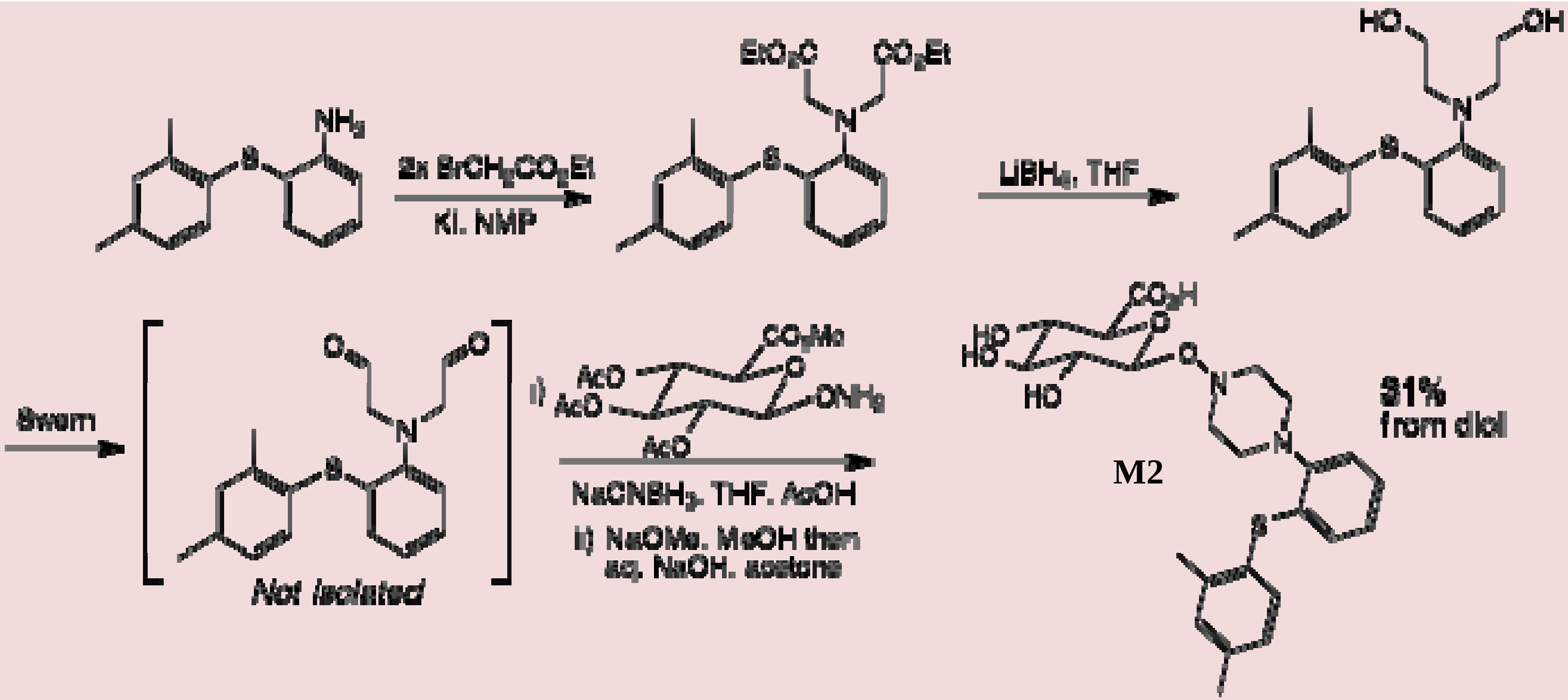
Glucuronide Synthesis

Case studies of some more demanding examples

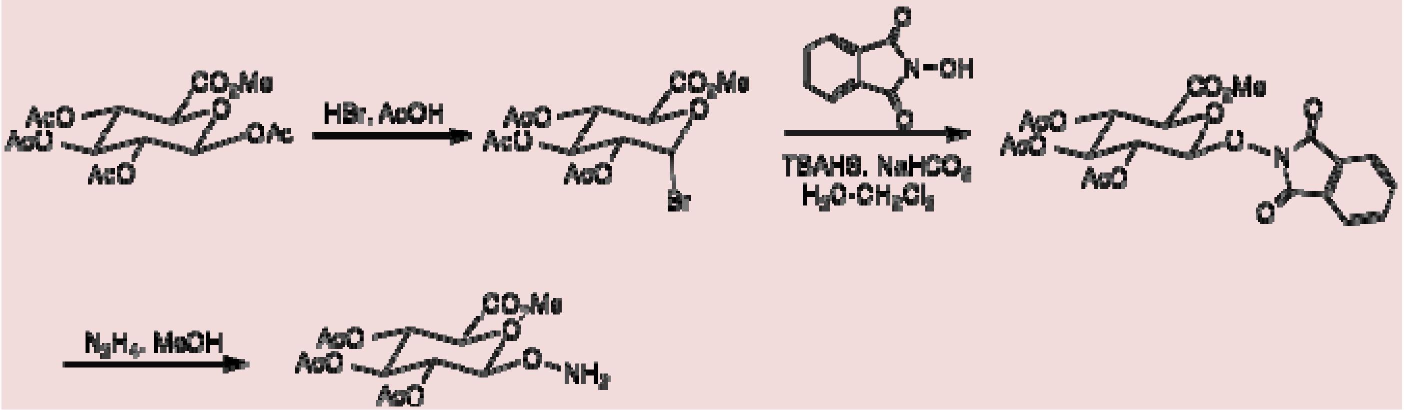
Lundbeck's antidepressant Lu AA21004 was metabolised, *via* the hydroxylamine, to a mixture of ***N-O*** and **quaternary ammonium** glucuronides:



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…

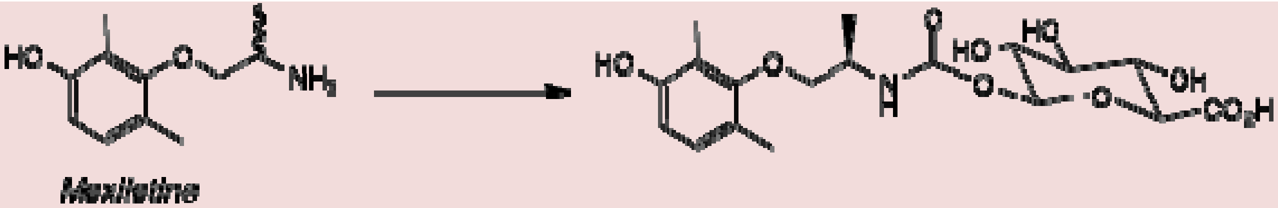


A modified Konigs-Knorr syntheis, with 2-phase alkylation, was very effective: brief deprotection selectively removed the phthalimide.

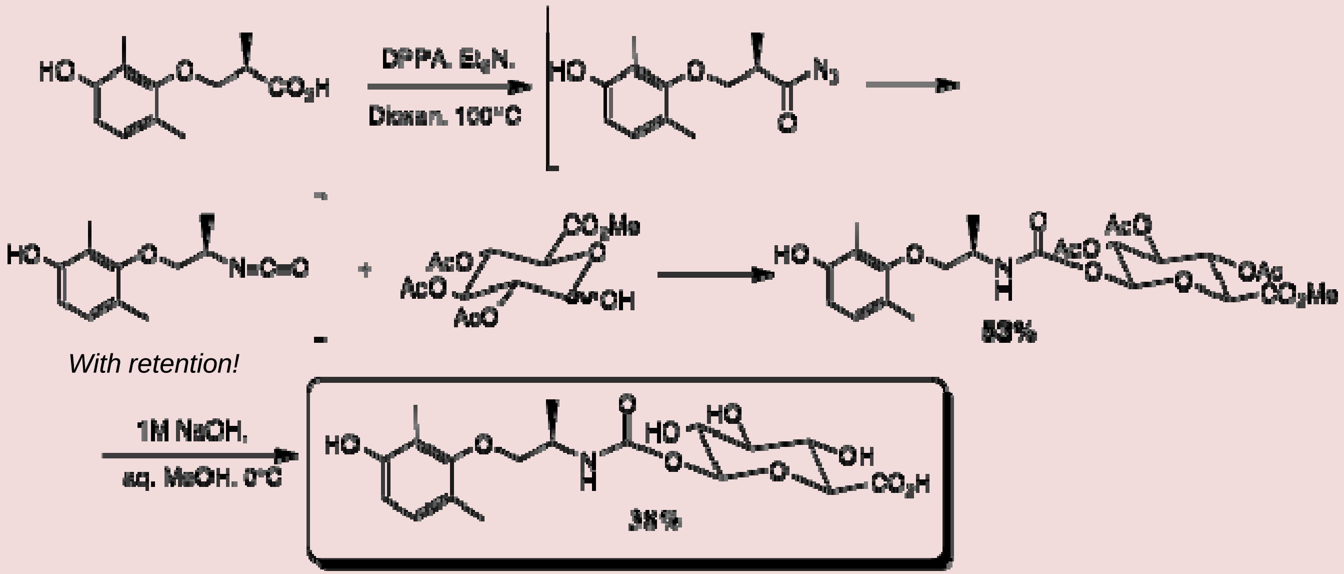
Drug Metab. Dispos. 2011, **39**, 2264-2274.

A carbamoyl glucuronide example

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



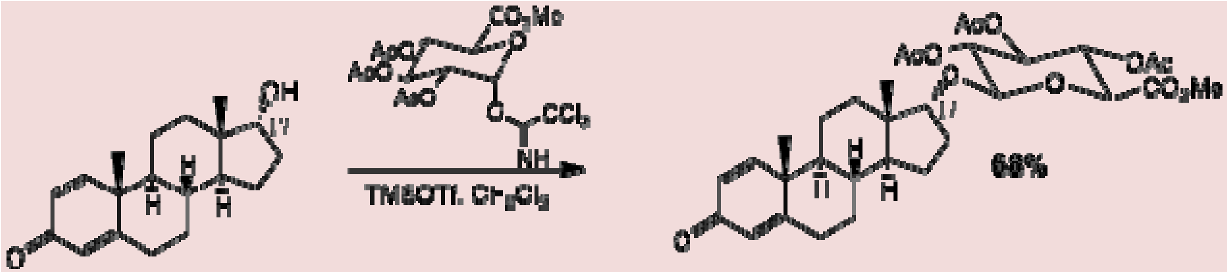
- Glucuronidation was highly enantioselective ➔ (R)-glucuronide
- Synthesis began with the (R)-carboxylic acid
- Unlike *O*-acyl glucuronides, carbamoyl glucuronides are stable to *brief* base hydrolysis



Tetrahedron Lett. 2010, **51**, 5265-5268

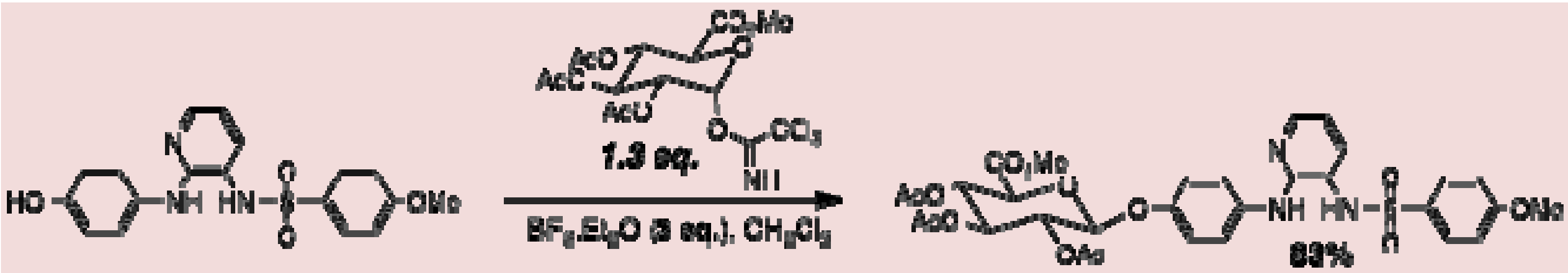
Imidate glucuronidation- often superior to Konigs-Knorr

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



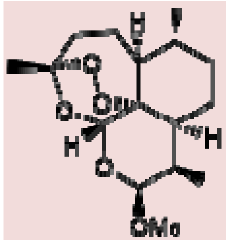
Here the Konigs-Knorr reaction failed- though it was fully satisfactory for the 17β-OH epimer.
Steroids 2009, **74**, 250-255.

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

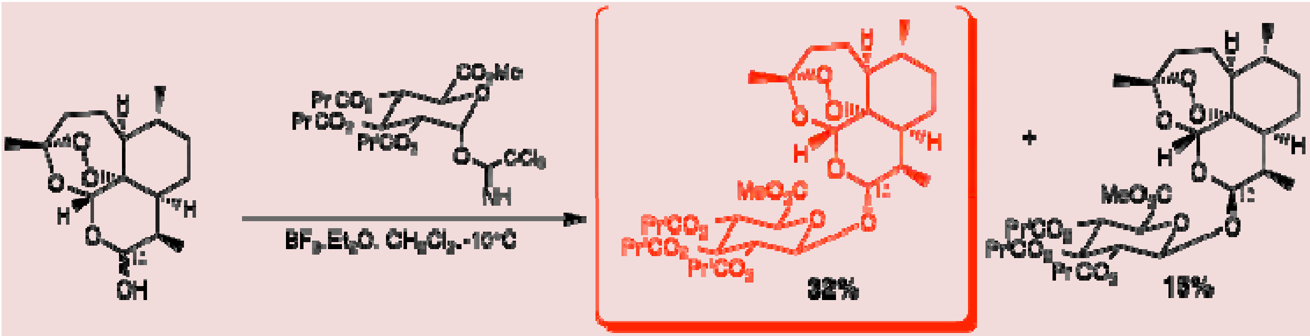


An earlier synthesis using the Konigs-Knorr reaction required *six* steps-
presumably the parent drug would not react.
Tetrahedron Lett. 2007, **48**, 1359-1362.

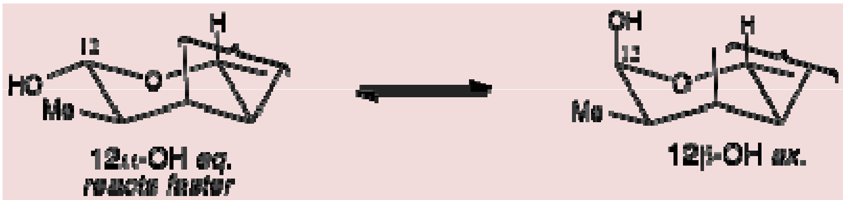
Semisynthetic artemisinins such as **arteemether** metabolise via CYP-mediated dealkylation, then glucuronidation. The human glucuronide proved to have 12α, 1´β- stereochemistry:



Artemether

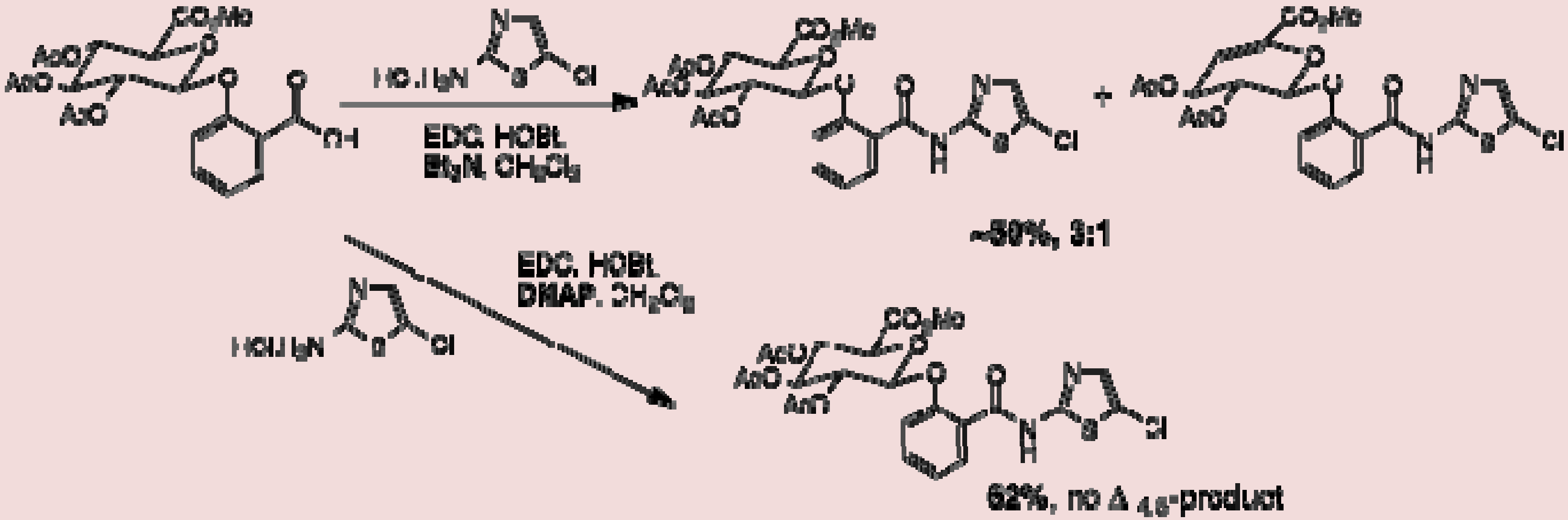


Conformational analysis of ring D of dihydroartemisinin:



Using *acetate* rather than *isobutyrate* protection, yield was <10%.
J. Med. Chem. 2001, **44**, 1467-1470.

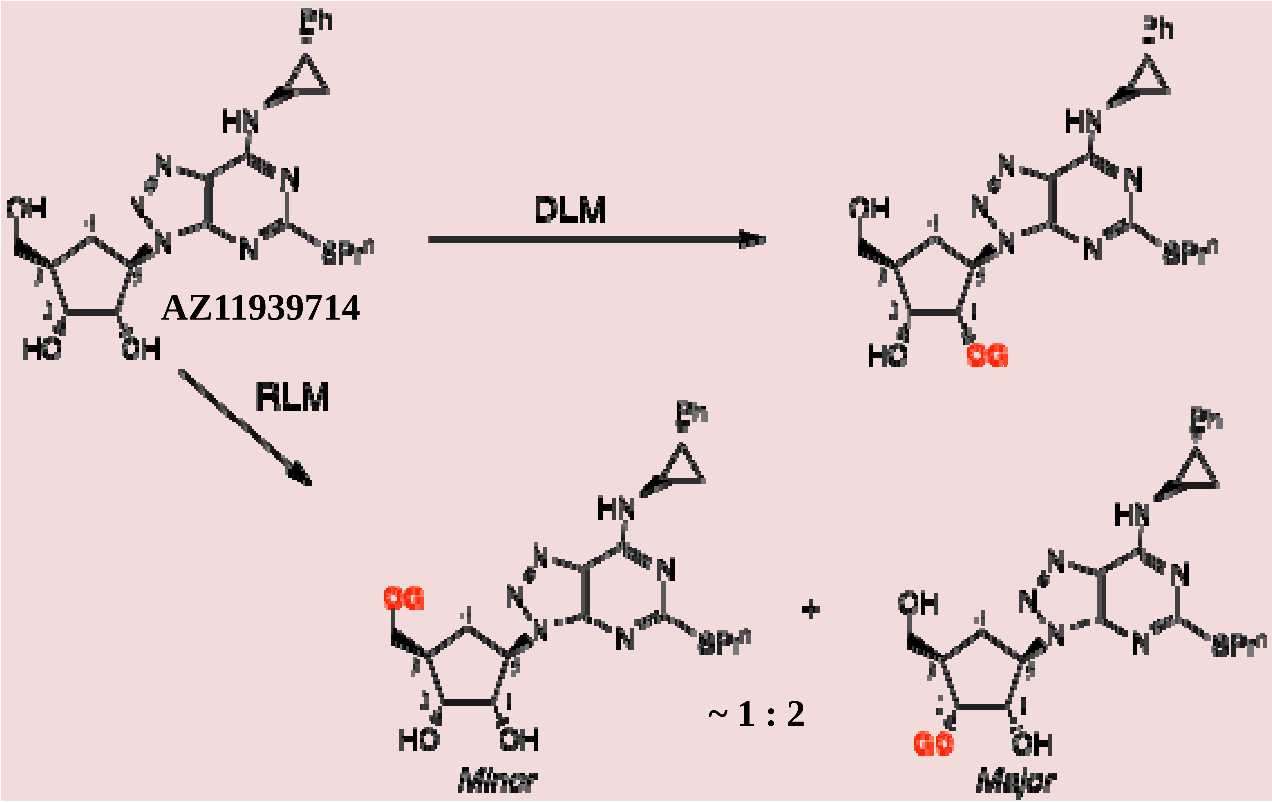
Beware *elimination* from glucuronides!-either in the final hydrolysis or whenever a strong base is used.



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

Enzymatic Glucuronide Synthesis

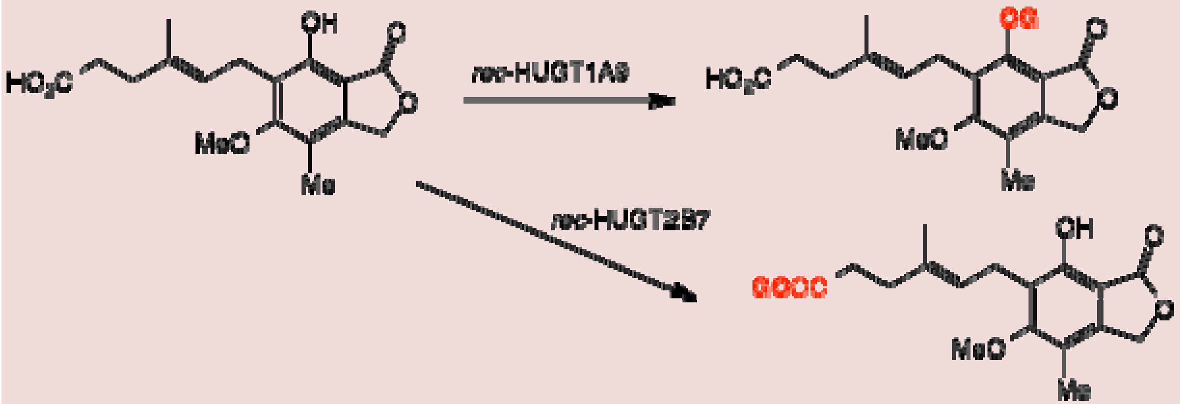
- Hepatic microsomes, recombinant human UGTs and engineered glycosynthases all offer possible routes-and may be regioselective in some cases.
- **Example-*species-selective* glucuronidation of an oral antithrombotic candidate:**



Cf. human liver microsomes: almost entirely the 3-glucuronide.
Drug Metab. Dispos. 2006, **34**, 1502-1507.

Human UGTs- site selectivity:

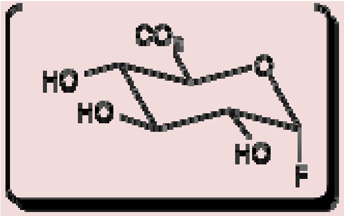
Mycophenolic acid- an important immunosuppressant, e. g. in transplantation:



…here again, the acyl glucuronide retains some on-target activity.
Drug Metab. Dispos. 2005, **33**, 139-146.

Use of an engineered glycosynthase

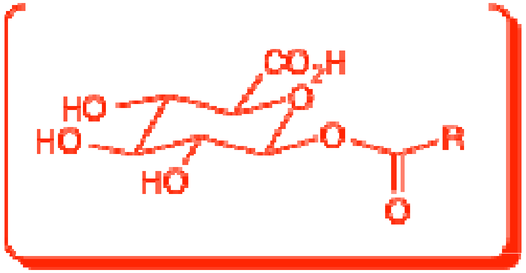
- Wild type glucuronidase ex *E. coli* (EC 3.2.1.31)
- Key mutation, Glu504 ➔ Gly/Ala/ Ser, abolishes hydrolytic activity
- A *glucuronyl fluoride* was used as the glycosyl donor
- A range of *O-alkyl glucuronides* made, generally good yields



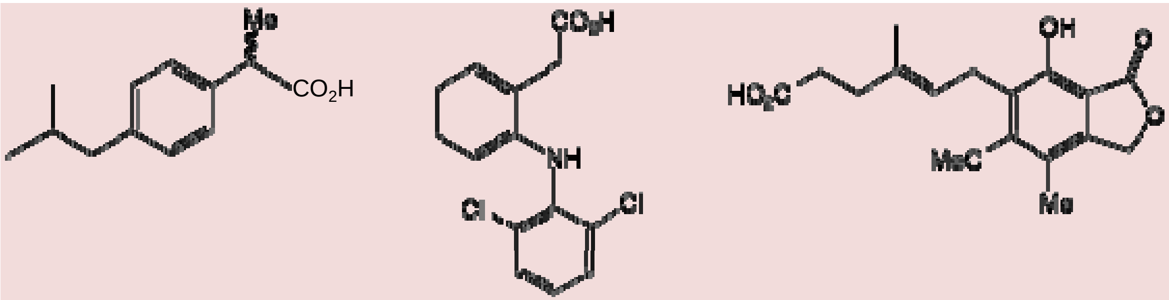
J. Org. Chem. 2011, **76**, 1992-2000

Acyl Glucuronides

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



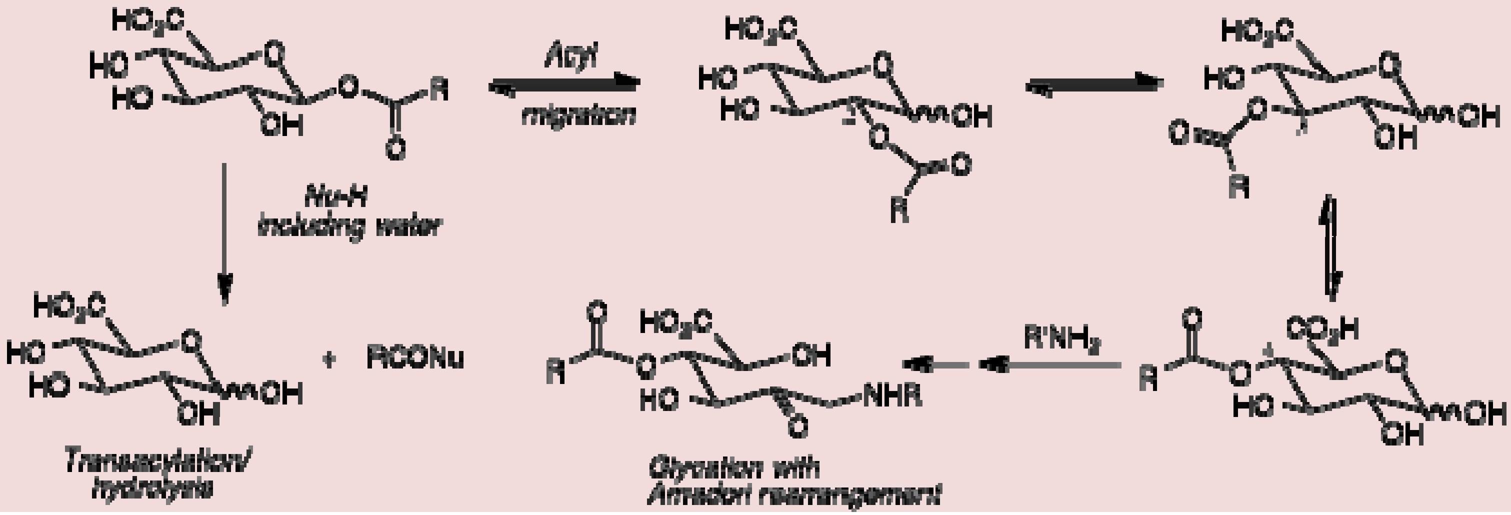
- Many well-known drugs, including NSAIDS, are significantly metabolised as acyl glucuronides-see below
- Considering first *chemical* reactivity, the nature of R is important:
- R = aryl: Reactivity predictable using Hammett considerations
- R = alkyl: Degree of α-substitution is very important…
  R = CH_2R', R = CHMeR', R = CMe_2R' show a clear gradation of properties.



Ibuprofen **Diclofenac** **Mycophenolic Acid**

Chemical Reactivity of Acyl Glucuronides

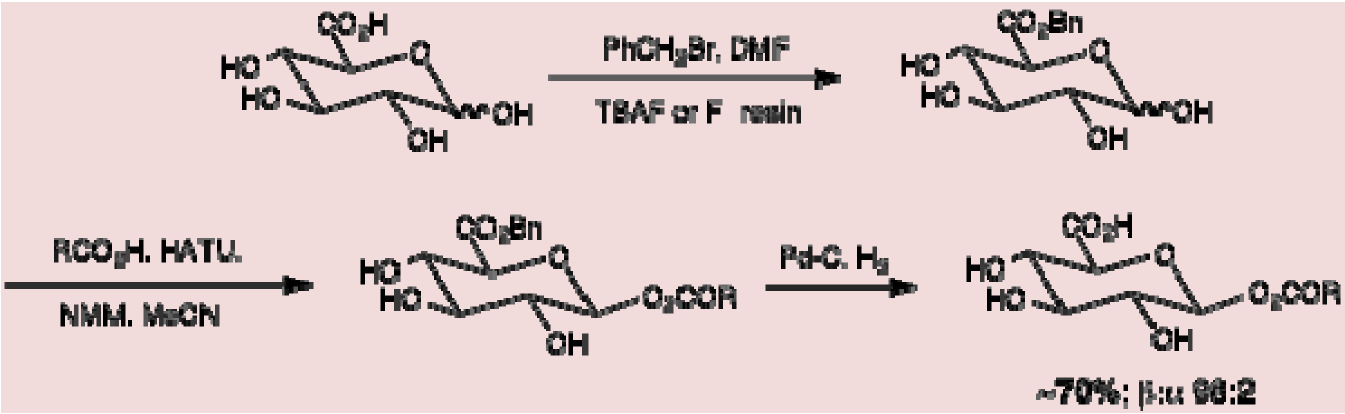
In addition to direct reaction with nucleophiles (**hydrolysis** or **amination** by e. g. lysine residues in proteins), acyl glucuronides may undergo **acyl migration** in a base-catalysed process…



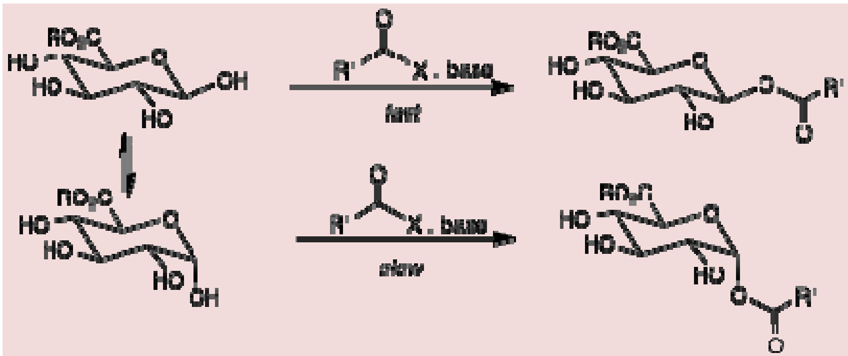
- Acyl migration is rapid in vitro (e. g. aq. buffer) at pH 6.5 or greater; $t_{1/2}$ increases with the degree of α-substitution , e. g. Me_2C> MeCH> CH_2.
- Transacylation (by external nucleophiles) is *significantly slower* for 2/3/4-*O*-acyl isomers
- The acyl migrated species may also react with nucleophiles, e. g. Lys-NH_2, by addition at C(1) followed by rearrangement (glycation-Amadori pathway)
- *Only* 1β-acyl glucuronides are good substrates for glucuronidases

Synthesis of O-Acyl Glucuronides

Using minimal carbohydrate protection and highly selective acylation, a wide range of 1β-*O*-acyl glucuronides can be prepared with excellent stereoselectivity…



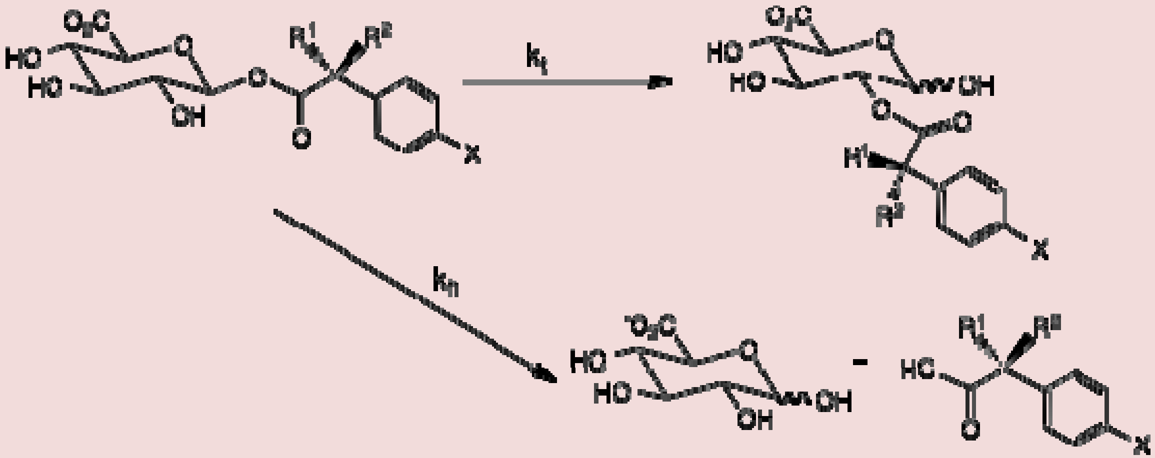
Allyl and PMB esters are also useful; compatibility of functionality in R will decide which is appropriate.
Org. Lett. 2005, **7**, 2591-2594; *Tetrahedron* 2007, **63**, 7596-7805.



Kinetic anomeric effect

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, α-substitution is key.
- Composite reaction is a mixture of transacylation and hydrolysis:

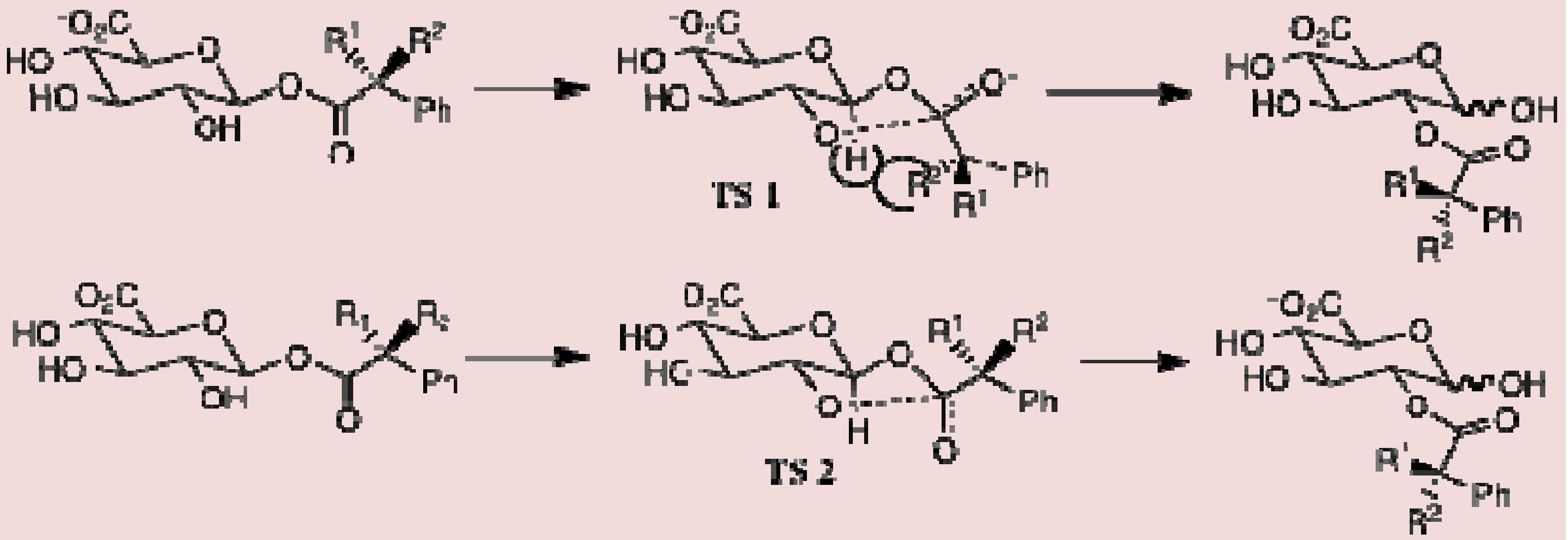


When X = H:

Acyl glucuronide	k_d h^{-1}	$t_{1/2}$ h
$R_1 = R_2 = H$	2.353	0.29
$R_1 = Me, R_2 = H$ (2R)-	0.903	0.78
$R_1 = H, R_2 = Me$ (2S)-	0.405	1.71
$R_1 = R_2 = Me$	0.029	23.30

In this series, k_h <10% of k_t.
To a good approximation, the first-order NMR degradation rate reflects k_t.
Org. Biomol. Chem. 2009, **7**, 2525-2533.

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



…the (2S)- isomer has to adopt the higher energy TS2.
Empirically the 'twofold rule' was well known.

Acyl glucuronides of Ibuprofen and derivatives

(R/S)-Ibuprofen **Ibu derivatives**

- The twofold R/S difference still applies: $t_{1/2}$s are **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:

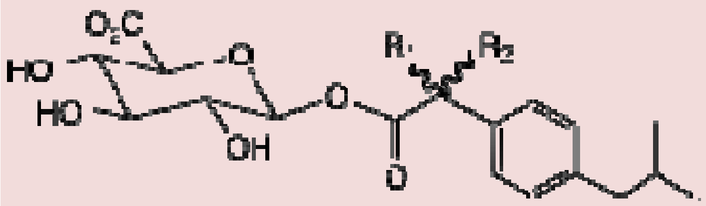
  $t_{1/2}$s = **7.24h** (ethyl), **9.24h** (allyl)
- For two significant *in vivo* metabolites (R = H):

  R_1 = OH, R_2 = H, $t_{1/2}$ = **5.03h;** R_1 = H, R_2 = CO_2H, $t_{1/2}$ = **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

Anal. Chem. 2007, **79**, 8720-8727; *Org. Biomol. Chem.* 2011, **9**, 926-934.

O-Acyl Glucuronides in Plasma

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



R_1 = R_2 =H, Ibufenac
R_1 = R_2 = H/Me, (R/S)-Ibuprofen
R_1 = R_2 = 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**, $t_{1/2}$s in **plasma** are :

Ibufenac	0.27 h
(R)-Ibuprofen	0.36 h
(S)-Ibuprofen	0.22 h
Bibufenac	5.2 h (2 μg/mL)

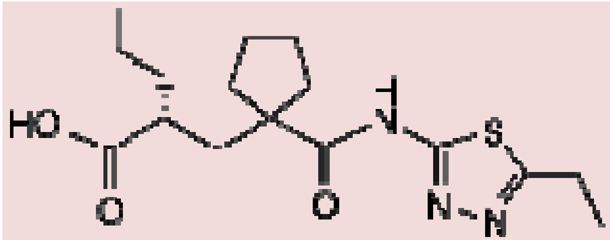
…from 5 to 18-fold lower.

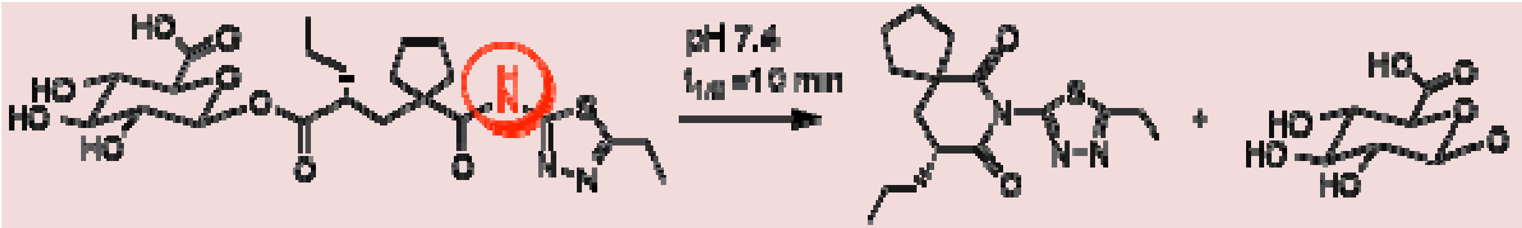
- HPLC-MS measurement necessary as NMR now impractical

Xenobiotica 2010, **40**, 9-23.

Case Study- An unusually reactive O-acyl glucoronide

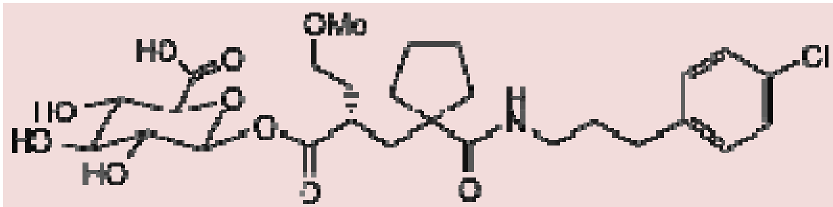
A candidate from a series of neutral endopeptidase inhibitors by Pfizer was withdrawn on toxicological grounds. Here the acyl glucuronide had a very short half life…





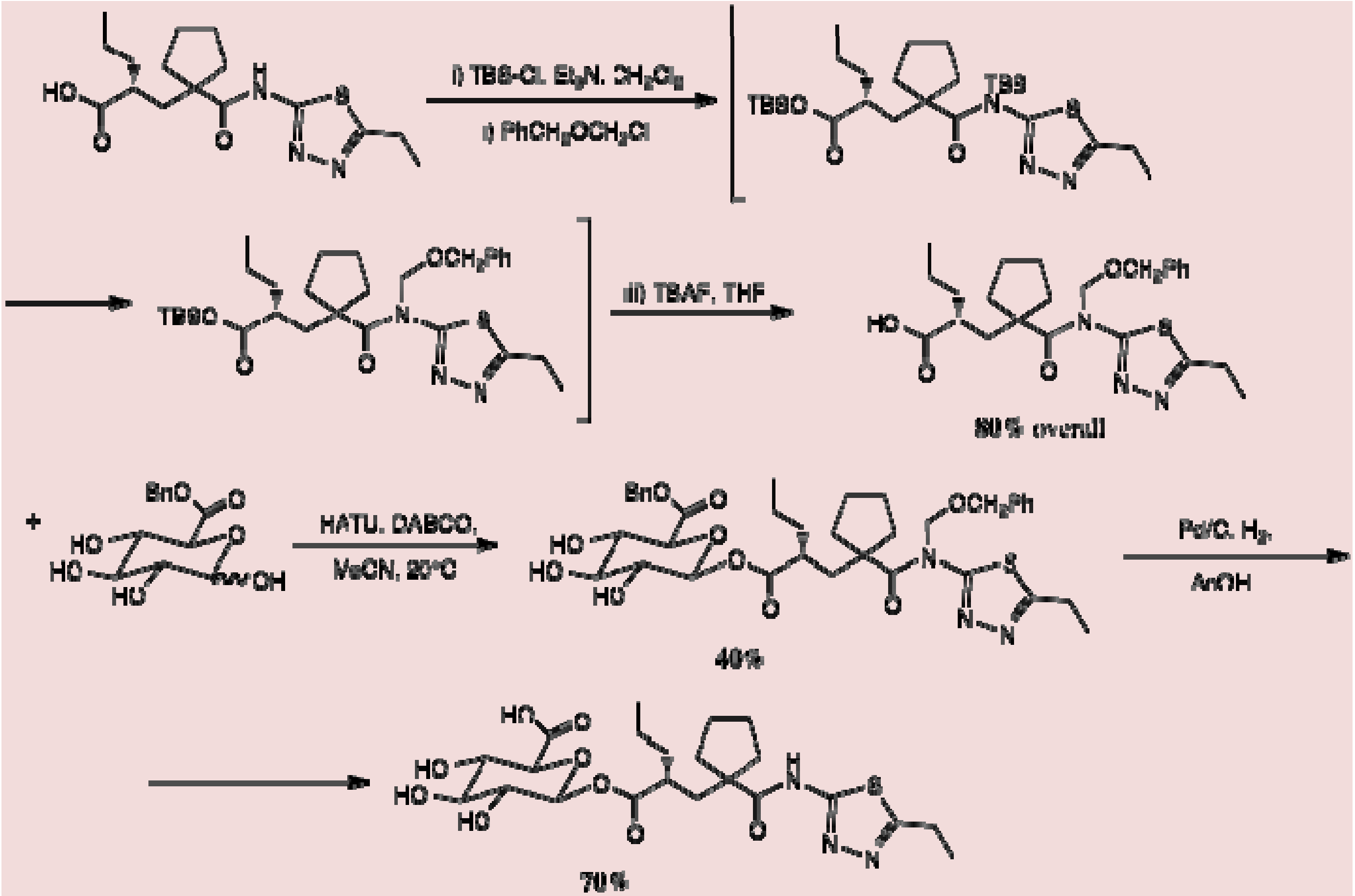
Culprit is the NH: pK_a ~ **9.5**.

Cf. another member of the series…



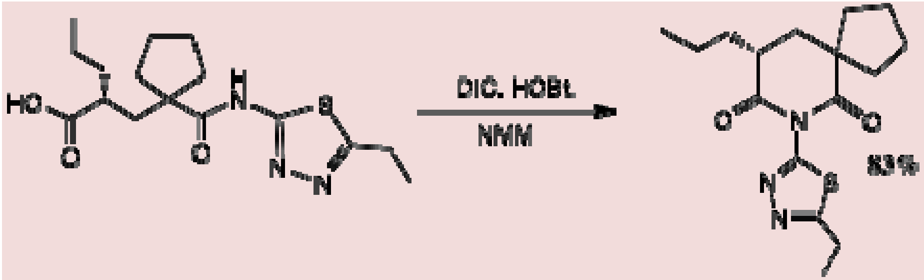
Here, pK_a (NH) ~ **16**: the acyl glucuronide has $\mathbf{t_{1/2}}$ **~50 h**.

Preparation of the acyl glucuronide required NH protection:

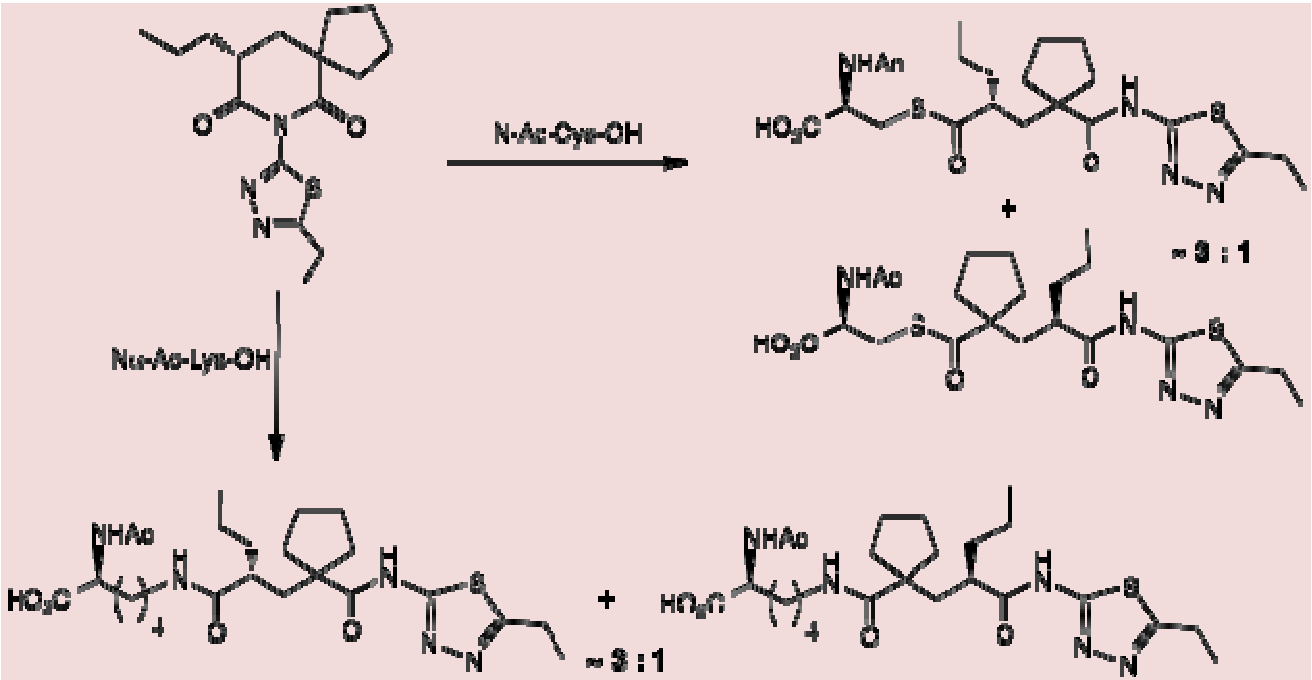


- The benzyloxymethyl (Bom) group has been used in peptide synthesis
- Other N-protection (allyl, Boc, Z) not satisfactory
- The final AG is highly stable at pH ~ 3; rapidly cyclises at < physiological pH

The cyclic compound (glutarimide) was easily made independently:

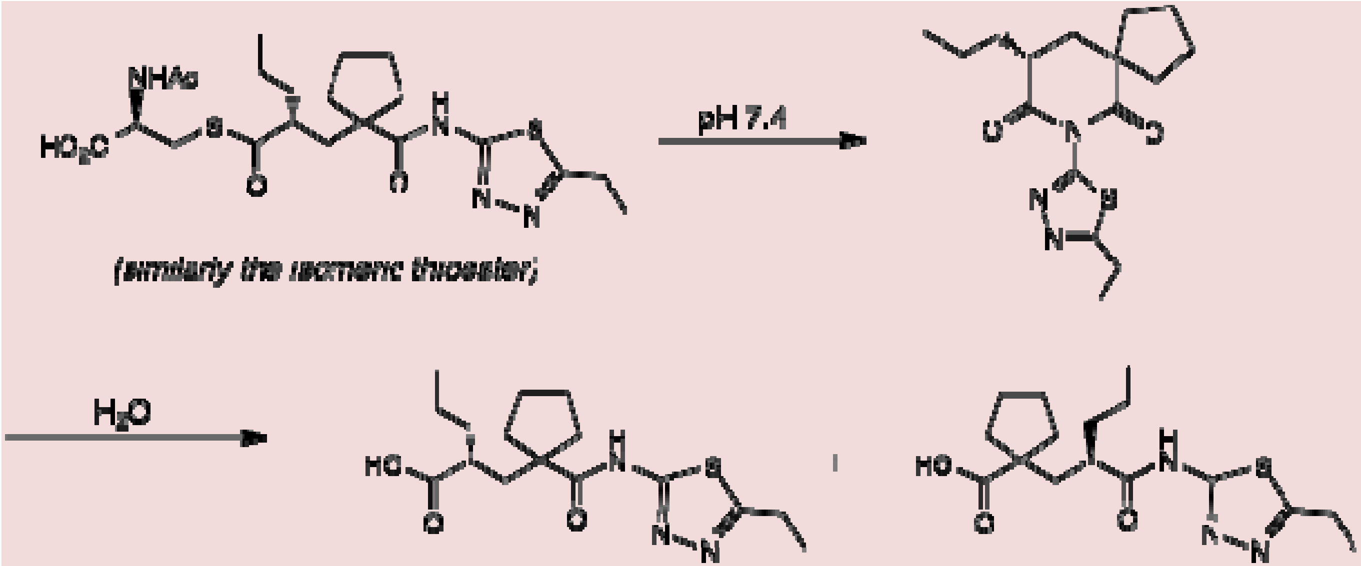


…as expected, it reacts readily with nucleophiles:



▪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.

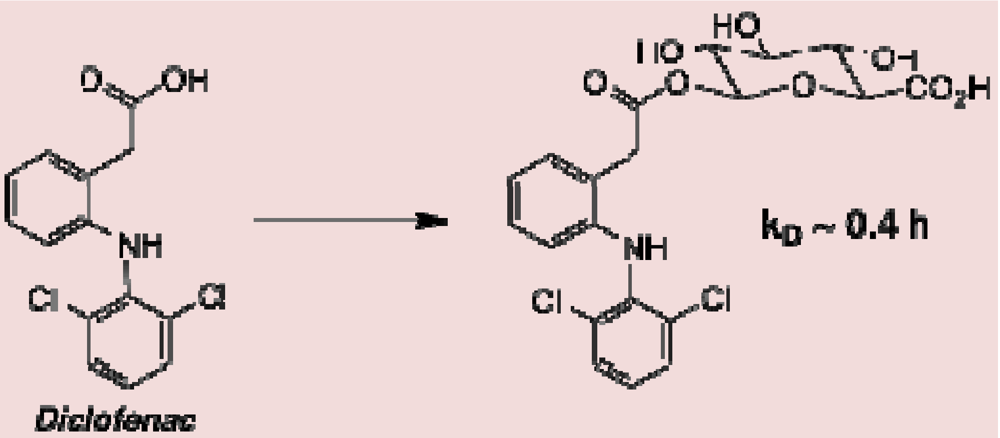


▪The **acyl glucuronide** showed similar reactivity to the imide with nucleophiles including hydrolysis

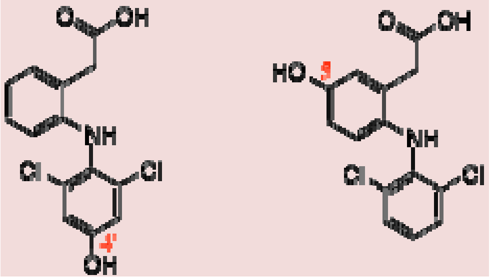
▪…we concluded that intermediacy of the **imide** explained the reactivity of the AG

J. Med. Chem. 2007, **50**, 6165-6176

Proteomics of Diclofenac Acyl Glucuronide



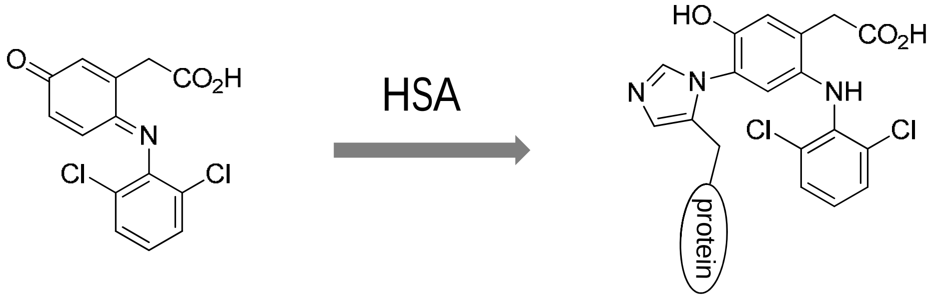
- Diclofenac AG has a short half-life and is known to be protein reactive *in vitro*
- We sought protein adducts of the AG in diclofenac patients *without* adverse drug reactions
- Other important diclofenac metabolites are the 4'- and 5-hydroxy derivatives:

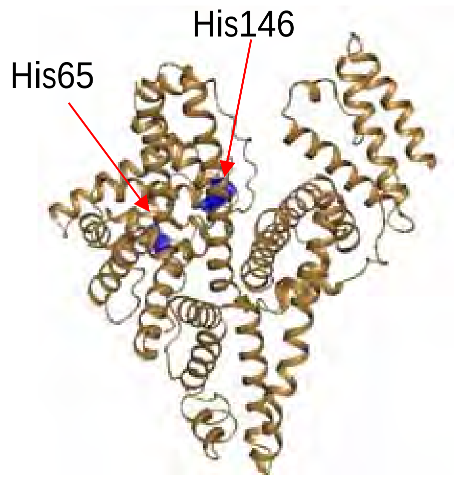


…both are oxidised to quinoneimines which can deplete glutathione
J. Med. Chem. 2004, **47**, 2816-2825.

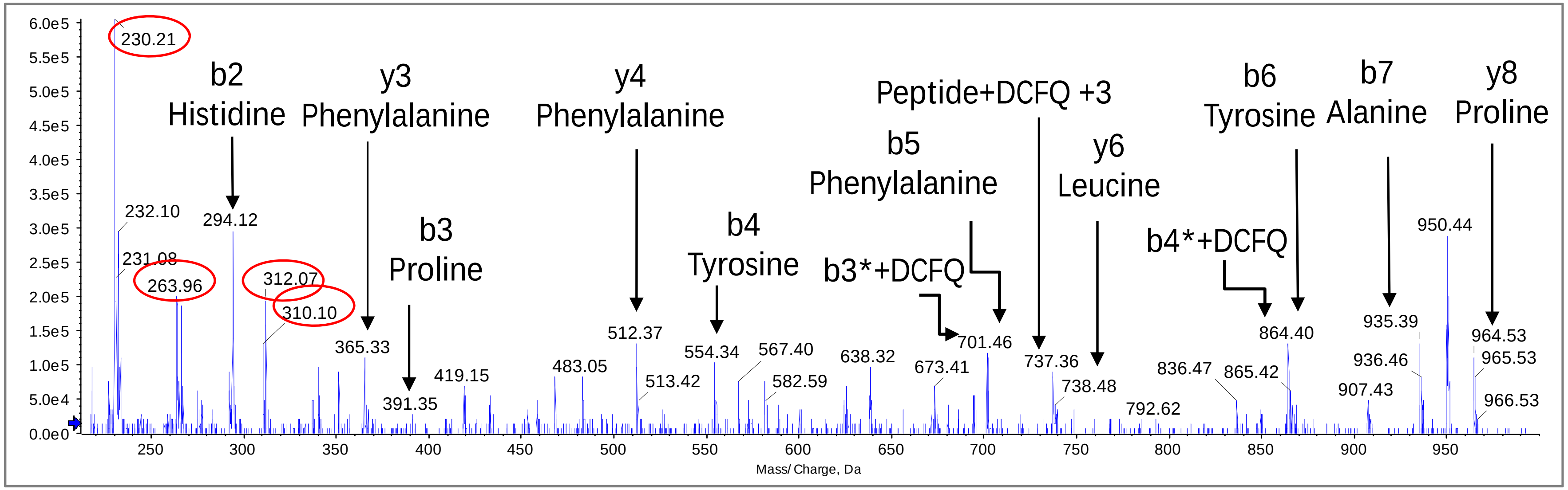
Diclofenac quinone imine forms albumin adducts *in vitro*

NB: the **5-OH** metabolite is the more readily oxidised; the derived QI is shown here.

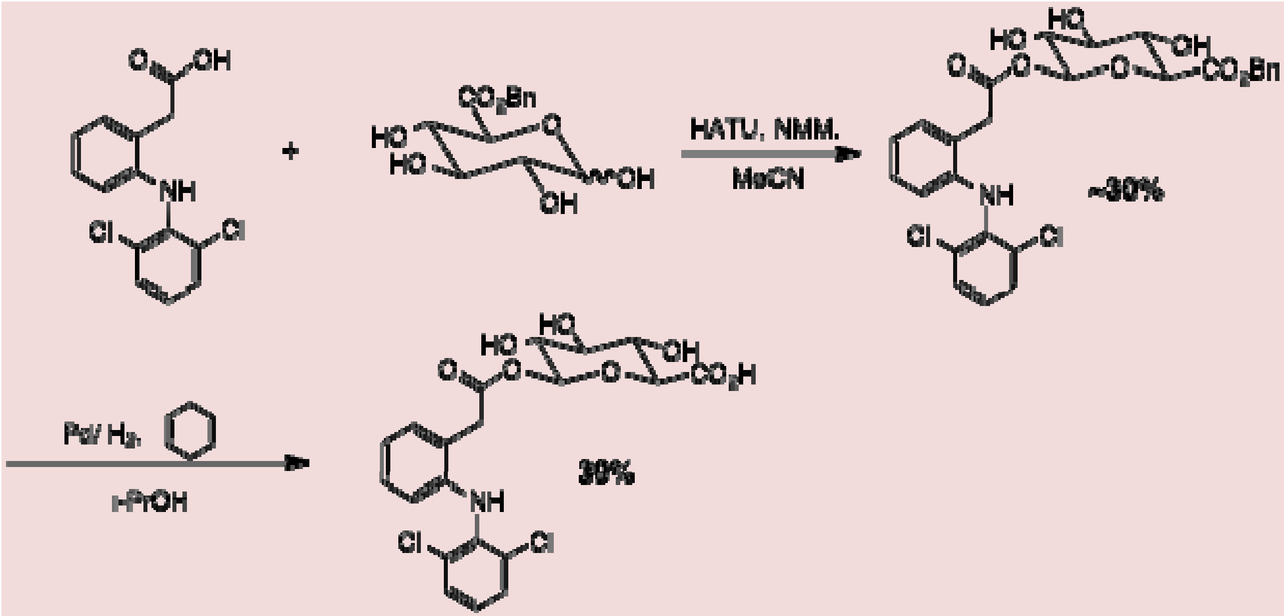




RH(DCFQ)PYFYAPELLFFAK



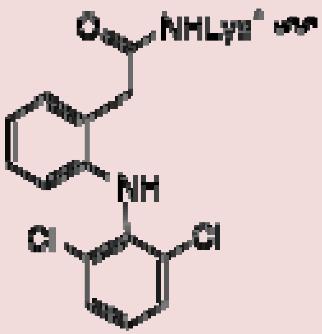
Synthesis of diclofenac AG:



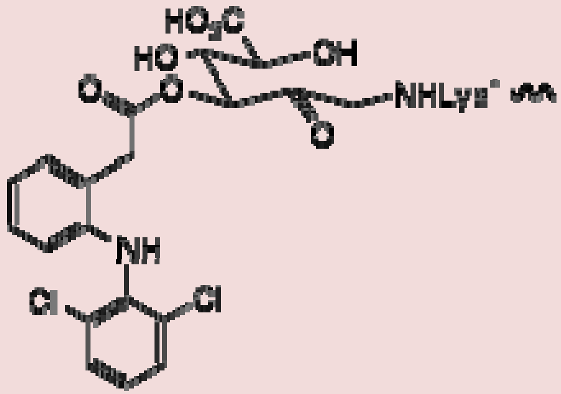
…we obtained a batch of 550 mgs, pure 1β-anomer, for the *in vitro* studies.

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:



Acylated adduct
(? possibly + Ser/Cys adducts)



Glycated adduct

- 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

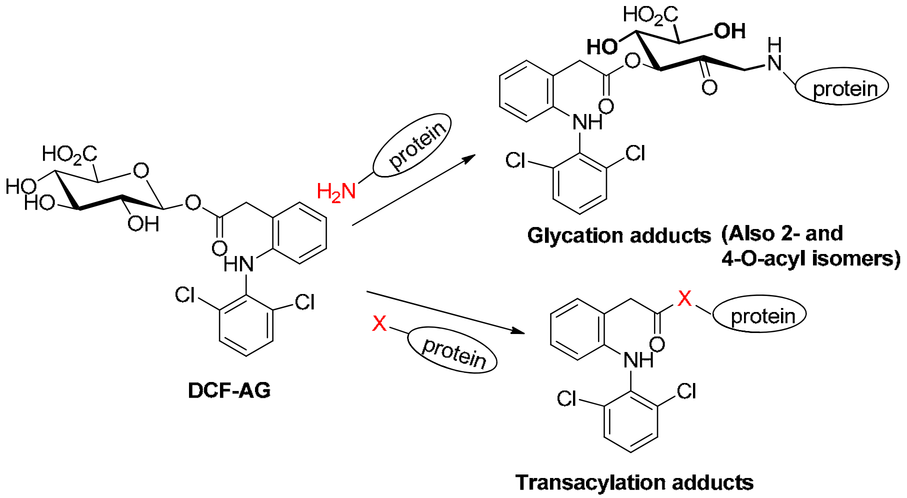
In vivo protein reactivity: Clinical plasma samples

- 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

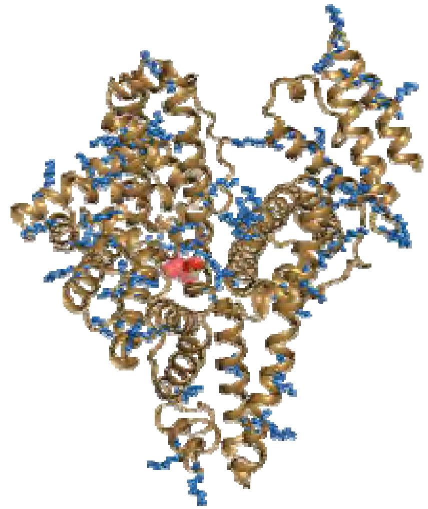
J. Pharmacol. Exp. Ther., MS in preparation

Structures…

Diclofenac forms albumin *glycation* adducts in man

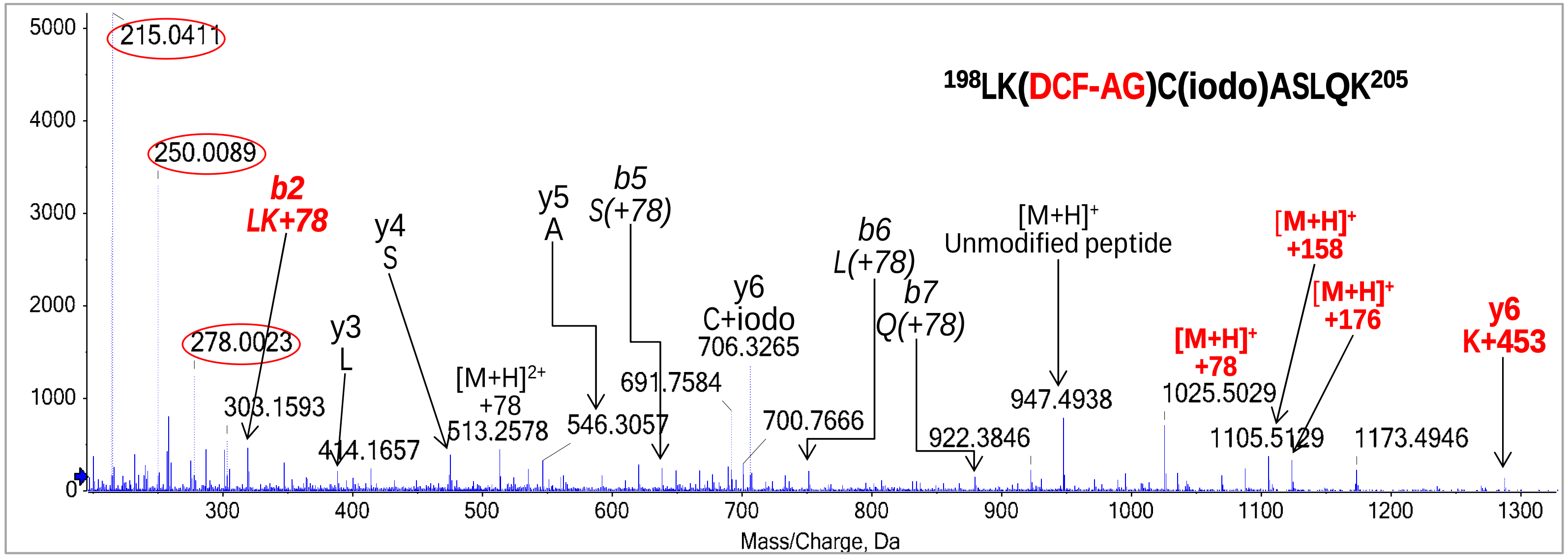


X=S, O, NH

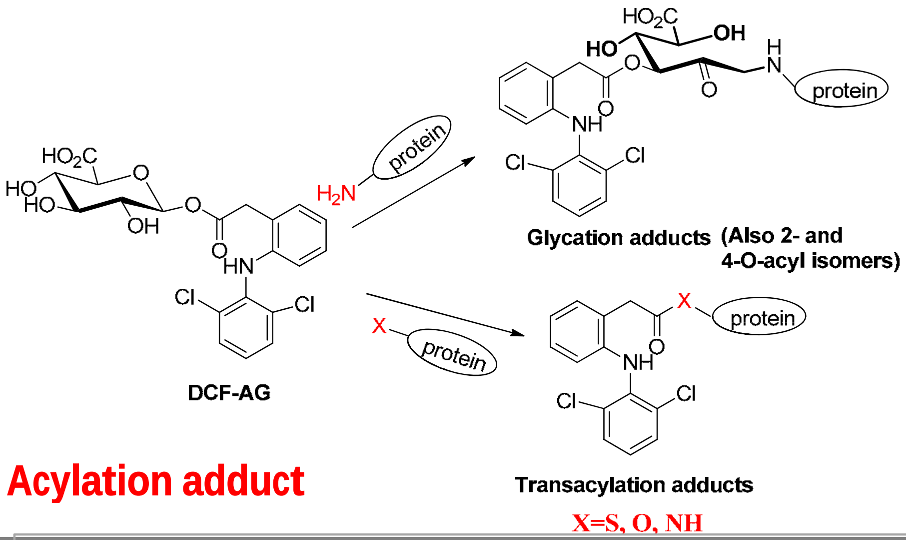


DCF-AG-Lys199 adducts detected in man

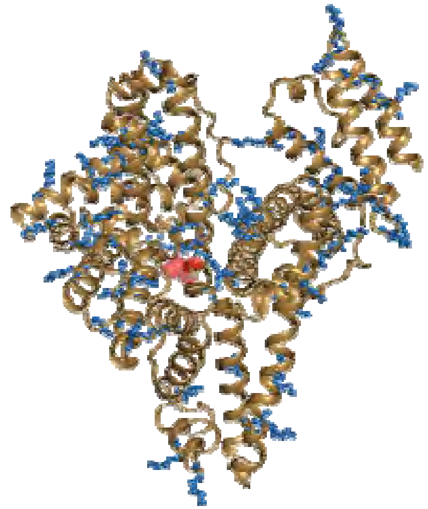
Glycation adduct



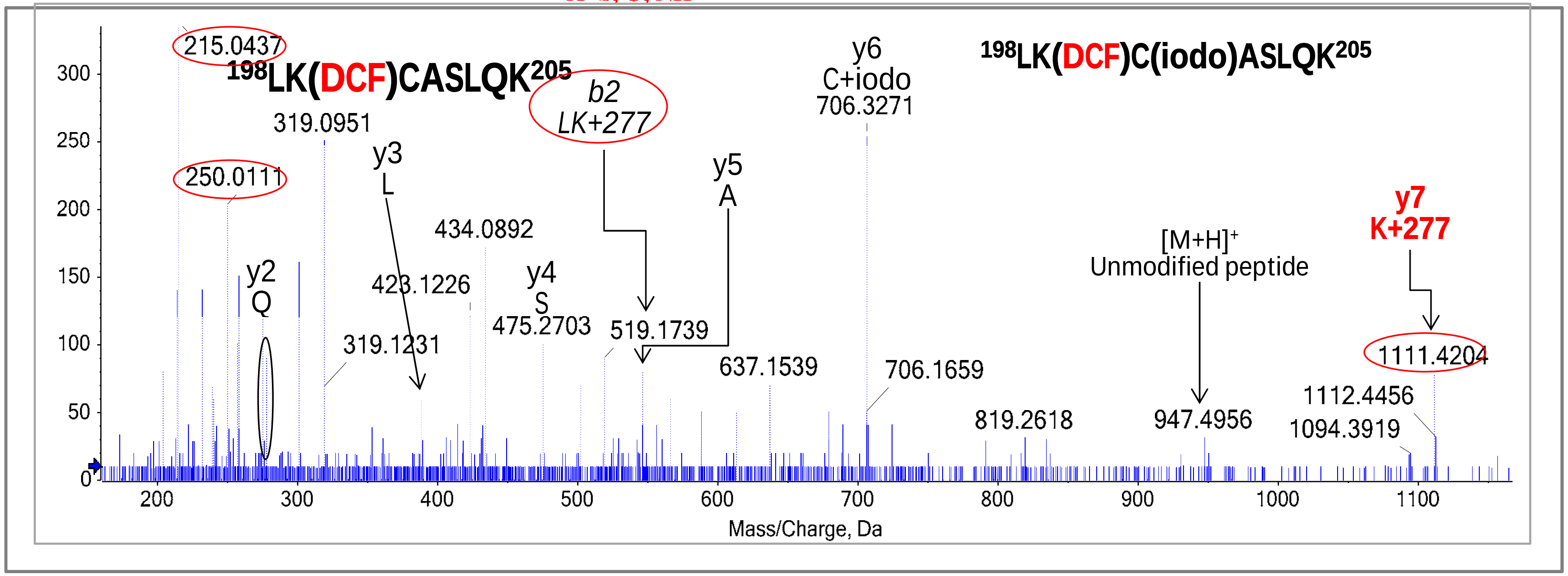
Diclofenac forms albumin *acylation* adducts in man



Acylation adduct



DCF-AG-Lys199 adducts detected in man



Acknowledgements

University of Liverpool

Chemistry

Jennifer Perrie
Xiaoli Meng
Lisa Iddon
Liz Bowkett
Amy Jones
Mazhar Iqbal
Neil Berry
Helen Wilson

University of Liverpool

Pharmacology and Centre for Drug Safety Science

Kevin Park
James Maggs
Rosalind Jenkins
Sophie Regan
Dom Williams

Astra Zeneca

Ian Wilson
John Harding
Gerry Kenna
Tom Hammond

Pfizer

Kevin Beaumont
Christopher Kohl
David Pryde
Simon Planken
Torren Peakman

Imperial College

John Lindon
Jeremy Nicholson
Toby Athersuch
Selena Richards
Caroline Johnson
Steve Vanderhoeven

UNIVERSITY OF LIVERPOOL