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Effective Synthesis of 1β-Acyl Glucuronides by Selective Acylation

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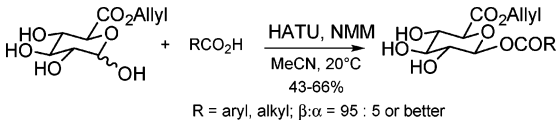
ABSTRACT

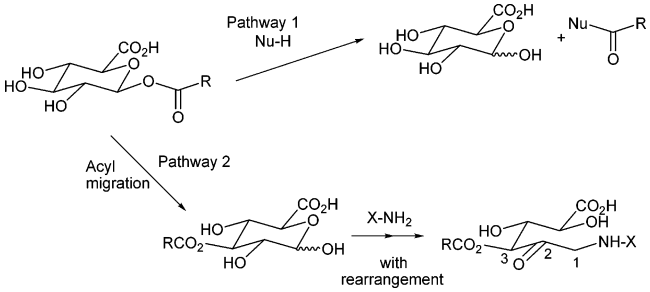


Acyl glucuronides are vital metabolites for many carboxylic acid containing drugs. We report an efficient new method for the chemical synthesis of these molecules by selective 1β-acylation of allyl glucuronate with carboxylic acids catalyzed by HATU and then mild deprotection through treatment with $Pd(PPh_3)_4$ and morpholine. The method is effective for a range of aryl and alkyl carboxylic acids, including important drugs.

Glucuronidation is a vital phase 2 metabolic process[1,2] whereby a wide range of drugs and xenobiotics may be rendered water-soluble, detoxified, and excreted. While *O*-glucuronides of alcohols and phenols are well-known and generally behave as stable organic molecules, acyl (ester) glucuronides of carboxylic acids have received less attention. Typically, acyl glucuronides are much less stable than those of alcohols or phenols, being subject to facile reaction with nucleophiles and rearrangement at acidic or basic pH (Scheme 1).[3]

Many important drug classes, notably the widely used nonsteroidal antiinflammatory drugs (NSAIDs), contain carboxylic acids and are metabolized as their acyl glucuronides. Whether acyl glucuronides cause adverse reactions—immune response or direct toxicity—is still a matter of lively debate.[4] For instance, the current consensus is that the acyl glucuronides of ibuprofen **1**[5] and naproxen **2** are benign, whereas that of diclofenac **3**[6] is suspect. It has been shown

Scheme 1. Reactions and Rearrangements of Acyl Glucuronides[a]



[a] Pathway 1: direct acylation ("glycation"). Pathway 2: Amadori rearrangement. The nucleophilic residues may be those present in body proteins.

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that some acyl glucuronides, e.g., that of diflunisal **4**, can cause binding of the parent drug to plasma protein (Figure 1).[7]

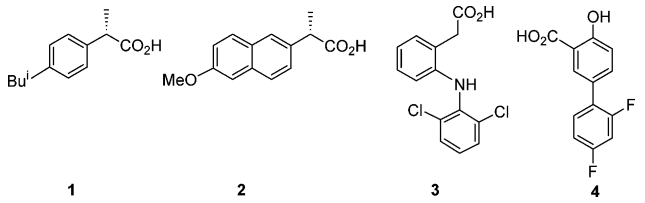


Figure 1. NSAIDs metabolizing as their acyl glucuronides.

As shown (Scheme 1), both direct acyl transfer (hydrolysis or reaction with other nucleophiles) and acyl migration followed by imine formation, then tautomerism (Amadori rearrangement) can occur. Recently, there has been an attempt to quantify the acyl donor ability of acyl glucuronides compared to other bioconjugates such as thioesters.[8]

It is therefore important to synthesize acyl glucuronides pure and in quantity as single 1β-anomers, as they occur in vivo, for thorough evaluation. Previously, two main approaches have been employed: either a fully protected derivative such as **5** (Figure 2) has been prepared, followed

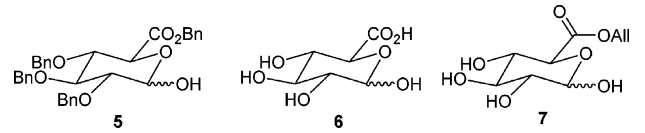


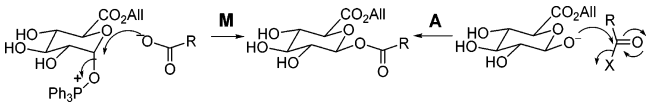
Figure 2. Glucuronic acid intermediates. All = $CH_2CH{=}CH_2$.

by conjugation to the carboxylic acid (e.g., via the imidate[9] method), or unprotected glucuronic acid **6** has been used. Intermediates such as **5** take many steps to prepare, and the use of unprotected glucuronic acid has been restricted to some special cases, e.g., retinoic acids.[10]

A promising alternative is to employ a monoester of **6**, namely allyl glucuronate **7**. Owing to the greater reactivity of the anomeric hydroxy group, **7** will react with carboxylic acids in a Mitsunobu reaction[11] to deliver fair yields of the desired conjugates. Deprotection with $Pd(PPh_3)_4$ then releases the free acyl glucuronide. We have employed this method to synthesize up to 200 mg of the acyl glucuronide of diclofenac **3**, but our experience[12] emphasized some shortcomings of the method. In particular, β/α mixtures from 5:1 to 2:1 were obtained in all cases[11] (we observed 4:1[12]). Satisfactory purification required both column chromatography and preparative HPLC: our best yield was 20%.

We considered that instead of the Mitsunobu reaction, proceeding ultimately by an S_N2 reaction at the anomeric center,[13] selective acylation might be a superior method, exploiting the kinetic anomeric effect[14] to favor 1β-acylation, Scheme 2. There was some precedent in a report[15] of

Scheme 2. Acyl Glucuronide Synthesis via Mitsunobu (M) or Acylation (A) Routes



selective 1β-acylation of glucose derivatives using activated esters of the carboxy component. We now report the successful realization of this concept in an effective synthesis of several 1β-acyl glucuronides.

We simplified the synthesis of **7** by using a resin-bound fluoride base[16] instead of DBU.[11] This greatly eased the workup (we had found it very difficult to remove DBU traces completely) and afforded crystalline **7** as an α/β mixture in 70% yield, containing variable amounts (from 5 to 20%) of a coeluting impurity by comparison with previous NMR data.[11] The impurity does not affect the efficiency of the following steps or the purity of the final products; recrystallization from MeOH affords essentially pure **7**, whose preparation we are continuing to optimize.

We then studied various combinations of carbodiimides, active ester-forming reagents, and base catalysts to activate and couple the carboxylic acid component, using 4-bromobenzoic acid **8** as the model substrate (Figure 3). 1-Hydroxybenzotriazole (HOBt) in conjunction with DIC (superior to DCC) was effective: later we found that the uronium reagent HATU[17] was the reagent of choice.

In early experiments, the active ester was preformed and then reacted with **7** and a base catalyst in acetonitrile. We later found that combining all reagents from the start gave

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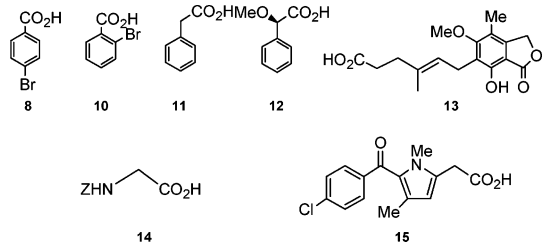

Figure 3. Carboxylic acids used in this study (with **1**).

an equally good yield. The pK_a of the base used proved critical: we found the optimum value was around 7.5 to 8. DABCO and *N*-methylmorpholine (NMM) were particularly effective, the latter being marginally superior. Weaker bases, e.g., pyridine, gave very slow reaction, while stronger ones, e.g., DMAP or triethylamine, led to overreaction.

The base was removed by neutralization with Amberlyst A-15 (H^+); the product was then isolated by filtration, evaporation, and silica gel chromatography. For the reaction of **8**, we obtained the desired product **9** in 52% yield using HATU (1 molar equiv) and NMM (2 molar equiv), with 1 equiv each of **7** and **8**, and then employed the method for a series of carboxylic acids **1** and **10**−**15**. Table 1 summarizes

Table 1. Yields and α/β Ratios of Acyl Glucuronides by the HATU−NMM Procedure[a,b]

				Mitsunobu method[11]	
carboxylic acid (equiv)	equiv of HATU, NMM	yield (%)	β/α ratio	yield[c] (%)	β/α ratio
8 (1)	1.2	52	19:1	48	5:1
8 (2)	2.4	59	β only		
1 (2)	2.4	65	β only		
10 (1)	1.3	52	β only	30	5:1
11 (1)	1.3	66	β only	31	3:1
12 (1)	1.3	65	β only	38	2:1
13 (1)	2.4	44	19:1		
14 (1)	1.2	43	β only		
15 (1)	1.3	52	β only		

[a] All reactions were carried out at 20 °×b0C in acetonitrile. [b] Reaction time of 2 h usually sufficient (monitored by TLC). [c] In the Mitsunobu procedure, 2 equiv of carboxylic acid was always used.

the yields obtained by the HATU procedure: using the HOBt-DIC method, yields were generally around 20% less. In many cases a very satisfactory yield of conjugate was obtained using just 1 equiv of HATU, but as noted in Table 1, the use of extra base or 2 equiv of HATU was beneficial in some cases, e.g., **8** (59%). It is also noteworthy that the presence of an ortho substituent, as in 2-bromobenzoic acid **10**, does not appreciably lower the yield, in contrast to the Mitsunobu procedure.

In all cases, the β/α ratio was excellent, 95:5 or better, the key ^{1}H NMR signal being that of the anomeric proton, typically δ 5.8 (1H, d, J = 7.8 Hz) for the β-anomer; any traces of α-anomer show δ 6.3 (d, J = 3.5 Hz).[3,18] As noted above, essentially pure β-product was obtained by a simple silica column; preparative HPLC was unnecessary.

Mycophenolic acid **13**[19] affords a most interesting example: this *Penicillium* metabolite has valuable antibiotic and immunosuppressant activity. We believe this is the first chemical synthesis of its acyl glucuronide, which had previously been obtained by preparative HPLC separation of an enzymatically synthesized mixture of its aryl and acyl glucuronides.[20,21] It was not necessary to protect the phenolic group in **13**, though the yield was slightly lower here than in most other examples.

In the cases of ibuprofen, (*S*)-**1**, and (*R*)-*O*-methylmandelic acid **12** the acyl glucuronides were formed as separable epimeric mixtures[22] in a 4:1 ratio. The most diagnostic ^{1}H NMR signals for the conjugates are those of the *CH*$_3$*CH*(Ar)-CO-unit for (*S*)-**1** and of the CH(OMe) unit for **12**. It is well-known that (*S*)-**1** racemizes at physiological pH. Zomepirac **15** is another significant clinical example: this antiinflammatory agent was withdrawn following serious allergic reactions[23] which, it was postulated, could be due to its acyl glucuronide.

Finally, we confirmed the deprotection procedure using Pd(PPh$_3$)$_4$ in conjunction with pyrrolidine[11] or morpholine[24] in THF: morpholine gave cleaner and higher yielding products. For example, using the Pd(PPh$_3$)$_4$−morpholine combination, the acyl glucuronide allyl esters of **11** and **13** were deprotected to afford the free acyl glucuronides **16a** and **16b** in 80% yield following brief silica chromatography (Figure 4).[25]

In summary, we have demonstrated a convenient synthesis of 1β-*O*-acyl glucuronides by selective acylation that admits a wide variety of structural types. Conditions are mild and

(16) Available from Sigma-Aldrich Co. Glucuronic acid in DMF was stirred with allyl bromide (1.1 equiv) and the resin-bound base at 40 °C for 16 h followed by filtration, evaporation and chromatography.

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(25) It was difficult to exclude completely small traces of Pd(PPh$_3$)$_4$ from the final product, even after chromatography. We have found very recently that a polystyrene-bound form of the Pd reagent (available from Argonaut Technologies Ltd.) affords reagent-free product at the ^{1}H NMR detection limit. The commercial resin was stirred in THF for 16 h, then filtered off and added to a solution of the allyl ester in THF containing morpholine (1 equiv) with stirring at 20 °C until complete deprotection was observed by TLC. Filtration, evaporation and chromatography (10−50% EtOH in CH$_2$Cl$_2$, silica) then afforded the free acyl glucuronide, e.g., **16a**.

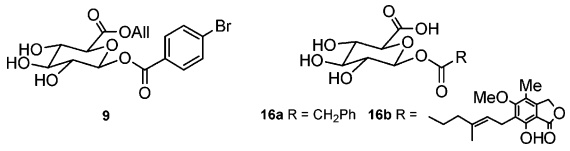

Figure 4. Acylation product and free acyl glucuronides after deprotection.

a satisfactory rate is obtained at 20 °C.[26] We believe this method should be extremely useful for the preparation of acyl glucuronide metabolites of many marketed and prospective carboxylic acid containing drugs.

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Supporting Information Available: Full characterization of new compounds described and copies of ^{1}H and ^{13}C NMR spectra of all compounds made. This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) **General Acylation Procedure.** 4-Bromobenzoic acid **8** (0.101 g, 0.5 mmol), allyl glucuronate **7** (0.117 g, 0.5 mmol), and HATU (0.190 g, 0.5 mmol) were stirred in dry acetonitrile (5 mL) with *N*-methylmorpholine (0.110 mL, 0.101 g, 2 equiv) under nitrogen at 20 °C. The reaction was monitored by TLC (10% EtOH−CH_2Cl_2, Merck Kieselgel analytical plates), and after 2 h it was quenched by addition of Amberlyst A-15 (H^+ form, 2 equiv). After evaporation at <30 °C (Buchi rotavapor), the residue was chromatographed on Merck 938 S silica, eluting with 7% EtOH−CH_2Cl_2. Appropriate fractions were pooled and evaporated, eventually under high vacuum, to afford the product **9**[11] as a foam (0.108 g, 52%).