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Synthesis and Biological Identification of the Acyl Glucuronide of the Antiinflammatory Drug ML-3000

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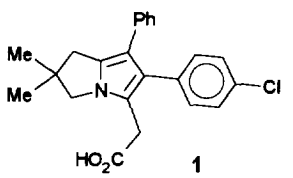
Abstract: The synthesis and identification in biological samples of the 1-*O*-acyl glucuronide **6** of the anti-inflammatory drug ML-3000 is described. Starting with D-glucuronic acid γ-lactone, 2,3,4-tris(*tert.*-butyl-dimethysilyl) glucuronic acid trichloroethylester **4** was prepared (in seven steps) and subsequently coupled with **1** under Mitsunobu conditions. Deprotection, *i. e.* removal of the trichloroethoxy group with zinc dust and desilylation with hydrofluoric acid in acetonitrile afforded a mixture of α- and β-**6** which could be separated by preparative HPLC. The abundance of **6** in bile and plasma samples obtained from animal studies with the cynomolgus monkey and the rabbit following repeated administration of **1** could be demonstrated by LC-electrospray MS analysis. © 1997 Elsevier Science Ltd.

The anti-inflammatory drug ML-3000 **1** is a non-redox dual inhibitor of both cyclooxygenase and 5-lipoxygenase.[1] The pharmacological properties of this compound were extensively characterized[2] and the safety profile allowed for initiation of the clinical development. In biological samples obtained from cynomolgus monkeys and rabbits the presence of an alkali-labile conjugate was detected. From



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HPLC/thermospray MS analysis a weak signal at m/z 556 was interpreted to be characteristic for the $[M+H]^+$-ion of the parent drug bearing a glucuronic acid moiety. UDP-glucuronosyl transferase mediated conjugation with D-glucuronic acid represents the major route for elimination and detoxification of drugs

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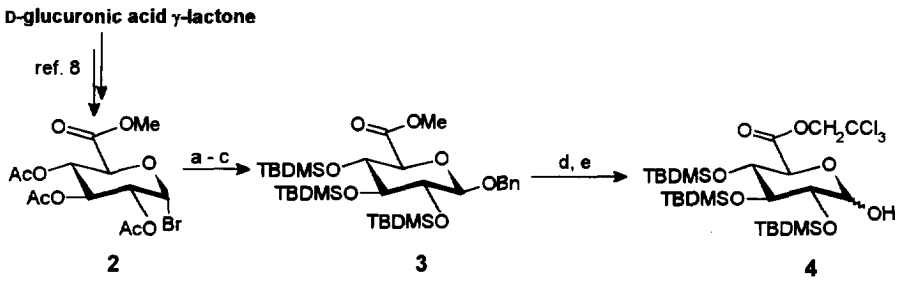
and endogenous compounds that possess a carboxylic acid group.[3] Due to the susceptibility to intramolecular and reversible acyl migration that is commonly found for these 1-*O*-acyl-β-D-glucuronides[4] and their high electrophilicity in reactions with protein-bound sulfanyl and hydroxy groups,[5] they are present in plasma in small concentrations only. In order to unequivocally confirm the proposed structure, the synthesis of **6** was initiated.

Synthesis

From earlier work it was known that ML-3000 is difficult to handle in various organic solvents like chloroform or ethyl acetate as well as under mildly acidic conditions (pH 5). *E. g.*, one common route of decomposition is decarboxylation. Hence, a synthetic strategy towards **6** had to take all these obstacles into account, including the ease of acyl migration of 1-*O*-acyl glucuronides. Although *Gygax et al.* had disclosed a straightforward multi-enzyme system with *in situ* regeneration of uridine-5′-diphosphoglucuronic acid for the synthesis of phenolic-*O*-β-D-glucuronides,[6] we preferred a chemical approach, thus avoiding lengthy optimization studies concerning the substrate specificity of UDP-glucuronyltransferase from guinea-pig liver homogenates toward ML-3000. Recently, *Tanaka* and coworkers[7] described a practical route to 1-*O*-acyl-D-glucuronic acids, using protective groups which are removed under neutral reaction conditions, thus making this kind of metabolites synthetically available.

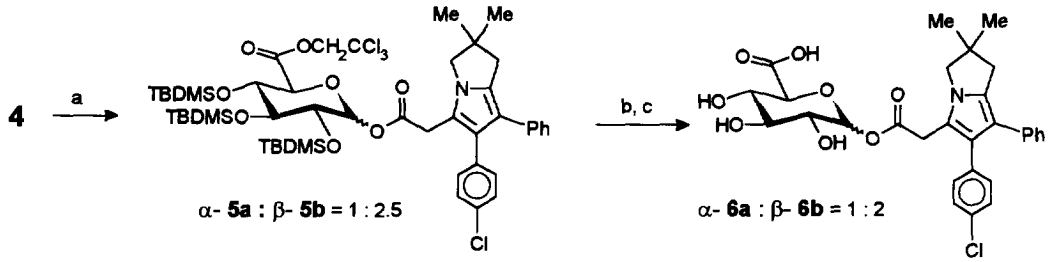
Scheme 1



Reagents and conditions: a) Ag_2O, BnOH, toluene, ms 4 Å, rt, 20h, 81 %; b) MeONa, $MeOH_{abs.}$, -40°C to 0°C, 12h, then Dowex-50W ($H^{\oplus}$); c) TBDMSCl, imidazole, 4-$DMAP_{cat.}$, DMF, 80°C, 48h, 56% from 2; d) 0.2 M NaOH, THF, 50°C, 10h, then HCl, workup, $(EtO)_2POCl$, Et_3N, toluene 0°C, 1.5h, then $HOCH_2CCl_3$, 4-DMAP, rt, 6h, 77%; e) Pd/C, H_2, EtOAc, rt, 48h, 71%.

In accordance to their work, the route starts from D(+)-glucuronic acid γ-lactone and follows the procedure reported by *Bollenback* and coworkers to afford bromide **2**[8] (Scheme 1). In the presence of freshly prepared silver oxide, **2** was converted into methyl(benzyl β-D-glucopyranosid)uronate under Koenigs-Knorr conditions in good yield. Deacetylation and silylation gave methyl ester **3** which was hydrolyzed under basic conditions, coupled with 2,2,2-trichloroethanol and transformed into derivative **4** by catalytic hydrogenolysis.[7]

Scheme 2



Reagents and conditions: a) PPh_3 (1 eq), DIAD, THF, 1 (2 eq), -50°C to rt, 2h, 72%; b) Zn, 1.0 M KH_2PO_4 buffer, rt, 12h, 67%; c) 40% HF, acetonitrile, 0°C to rt, 5h, 71%.

At this point there was uncertainty on whether acyl glucuronide formation with an amino acid like **1** can be achieved under Mitsunobu conditions. To our relief, coupling of glucuronic acid derivative **4** with ML-3000 **1** proceeded smoothly affording an anomeric mixture of acyl glucuronides **5** (α/β 1:2.5; colorless crystals: m.p. 82 °C (dec); $[\alpha]^{20}_D$= + 1.84 (c 0.87, MeOH)) (Scheme 2). The glycosidic bond in **5** turned out to be unstable. Thus, the acyl glucuronide could only be purified by flash column chromatography on silica gel in the presence of 0.05 % Et_3N or on basic Al_2O_3 (eluent: petroleum ether / ethyl acetate 20:1). At this stage, separation of both anomers was not possible. Therefore, the synthesis was continued with the α,β-mixture. Removal of the trichloroethyl group was accomplished using zinc dust in a buffered solution to yield the acid which was purified by flash column chromatography (eluent: $CHCl_3$ / MeOH 100:1, R_f = 0.36) and was further desilylated by hydrofluoric acid in acetonitrile without affecting the anomeric center. The target glucuronide **6** was isolated by flash column chromatography on silica gel (eluent: $CHCl_3$ / MeOH / HCO_2H 9:1:0.1, R_f = 0.31) as an amorphous powder (α/β 1:2; colorless crystals: m.p. 93 °C, dec. at 150 °C; $[\alpha]^{20}_D$= + 18.6 (c 1.05, MeOH)).[9] Both anomers were further separated and purified by HPLC [LiChrospher 100 C_{18}, 250 mm x 4 mm i.d.; flow: 1 ml/min; A: H_2O (pH 3.8 adjusted with formic acid), B: acetonitrile; gradient elution from A / B 60:40 to 5:95 within 45 min]. It is noteworthy, that 1-*O*-acyl glucuronide **6** did not undergo acyl migration under the reaction condition described nor during workup or chromatographic purification.

Identification of ML-3000 1-O-acyl glucuronide in biological samples

In biological samples hydrolysis of **6** was prevented either by cooling to approx. 8 °C or by slight acidification to pH 4 - 5. In Figure 1 chromatograms from analysis of bile and of the synthesized reference by HPLC-electrospray MS are presented. Recording of the prominent ions m/z 556 and m/z 380 demonstrates that only one glucuronide anomer is present in the biological sample, a result which is in accordance with the known exclusive formation of β-D-glucuronides. During prolonged storage of bile and plasma samples at -80 °C, a relevant formation of regioisomers or ester hydrolysis was not observed. The reactivity of this metabolite under physiological conditions will be subject to further investigations.

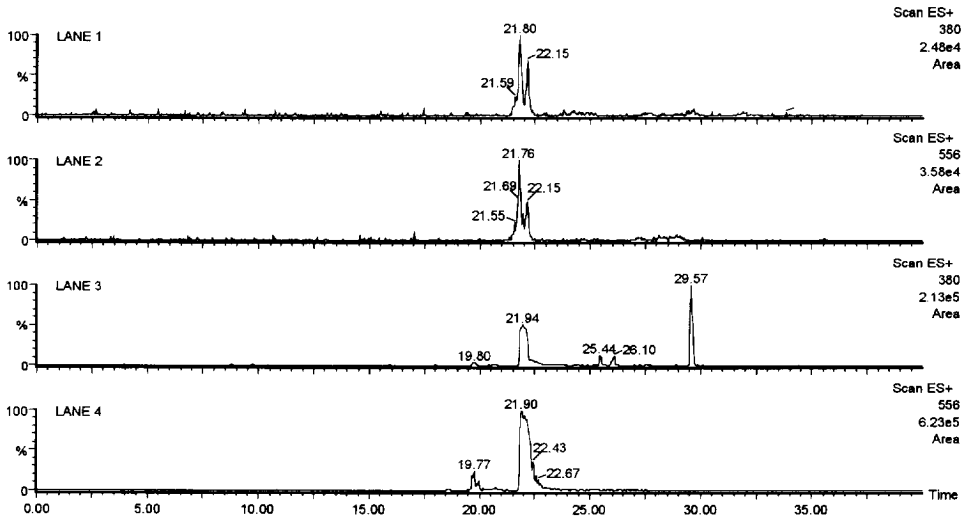


Fig. 1: HPLC-electrospray MS analysis of ML-3000 1-O-acyl glucuronide **6**; Lane 1 and 2: Separation of chemically synthesized α-/β-anomers (scan m/z 556 ($[M+H]^+$) and m/z 380 ([M+H-glucuronic acid]$^+$); Lane 3 and 4: Analysis of bile from cynomolgus monkey (scan m/z 556 and 380).

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(9) **6a** and **6b** were fully characterized by ^{1}H- and ^{13}C-NMR spectroscopy as well as by APcI-MS spectrometry.

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