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The synthesis of the glucuronide adduct of Trocade™

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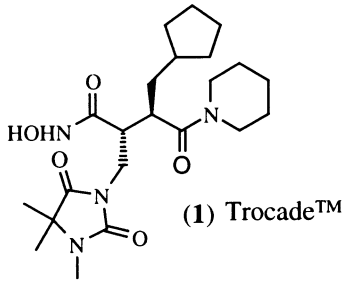
Abstract

The synthetic preparation of the glucuronide adduct of Trocade™, a selective inhibitor of the MMP collagenase, is described. This was achieved by preparation of the protected *O*-glucuronsyl hydroxylamine (**9**) and subsequent coupling to the available carboxylic acid (**7**), followed by deprotection. The synthetic material was identical to authentic isolated metabolite, which confirmed that glucuronidation of Trocade™ occurs on the oxygen of the hydroxamic acid. © 2000 Published by Elsevier Science Ltd.

Hydroxamic acids represent a class of compound which are currently of great interest with respect to inhibition of matrix metalloproteases (MMPs).[1] Trocade™ (**1**) is a selective inhibitor of the MMP collagenase, and is currently undergoing Phase III clinical trials for the treatment of rheumatoid arthritis (Fig. 1).



(**1**) Trocade™

Figure 1.

Studies on the metabolism of Trocade™ in marmosets and by using human liver slice microsomes indicated glucuronidation to be the predominant metabolic fate of the drug substance. Glucuronidation is a well-known drug metabolising reaction and is generally regarded as a 'detoxification' process, with the drug–glucuronic acid adducts usually being cleared renally.[2]

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Two structures were proposed for the glucuronide adduct (Fig. 2), one oxygen linked (**2**) and the other linked through nitrogen (**3**). These structures were based upon the following evidence, obtained from a sample of the metabolite isolated from marmoset urine:

- the m/z of the adduct was consistent with a glucuronide
- the ^{1}H NMR spectrum contained the elements of Trocade™ and glucuronic acid
- the molecule did not mutarotate, indicating the aglycone was coupled at the anomeric position
- the chemical shift ($\delta = 4.7$) and coupling constant ($J = 7.9$ Hz) of the anomeric proton in the ^{1}H NMR spectrum were consistent with a β-anomer
- treatment of the glucuronide with β-glucuronidase returned Trocade™. This supported coupling to the sugar through the hydroxamic acid linkage.

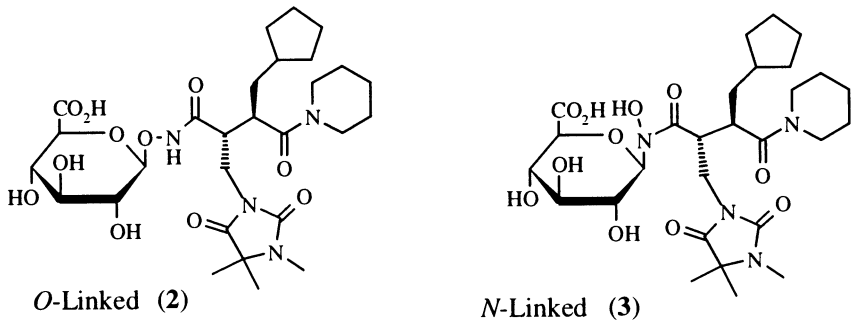
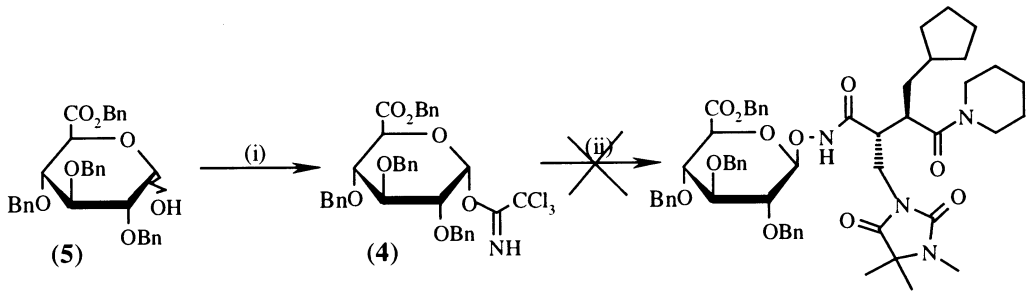


Figure 2.

Only a few μg of isolated metabolite were available at this time therefore further studies were not possible in order to distinguish between structures (**2**) and (**3**). A chemical synthesis was required to confirm unambiguously the structure of the glucuronide and also to provide an analytical reference sample of the metabolite.

Our initial efforts focused upon the Lewis acid mediated coupling of Trocade™ to the fully benzyl protected glucuronic acid trichloroacetimidate (**4**) (Scheme 1). This process is well precedented[3] for the synthesis of alkyl glucuronides, but has not been reported for hydroxamic acids. Additionally, the sugar (**5**) prepared as an anomeric mixture reacts with trichloroacetonitrile to furnish the trichloroacetimidate (**4**) exclusively as the α-anomer.[4] This in turn reacts with nucleophiles to afford predominantly β-glucuronide as required.



Scheme 1. *Reagents*: (i) trichloroacetonitrile; (ii) Trocade™/$BF_3{\cdot}OEt_2$ (cat.)/CH_2Cl_2

Despite several attempts, no coupling was detected when Trocade™ was used as the nucleophilic component; the hydrolysed sugar (**5**) was always obtained following work-up of the reaction mixture. Activation of (**5**) as a reactive sugar triflate was also unsuccessful, whilst the attempted coupling of (**5**) with (**1**) under Mitsunobu conditions degraded the aglycone. These results suggested the hydroxamic acid was not sufficiently nucleophilic to react with the activated glucuronic acid derivative. The literature also indicated that sugar uronates are generally poor glycosyl donors.[5] Thus we changed tack, and looked at the alternative disconnection depicted in Fig. 3.

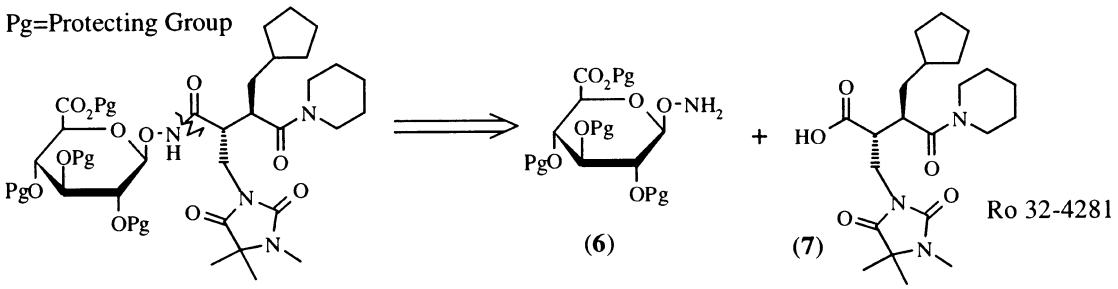
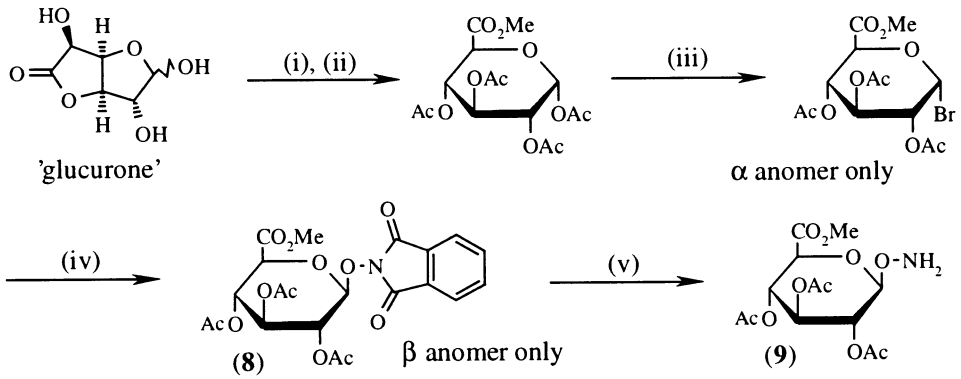


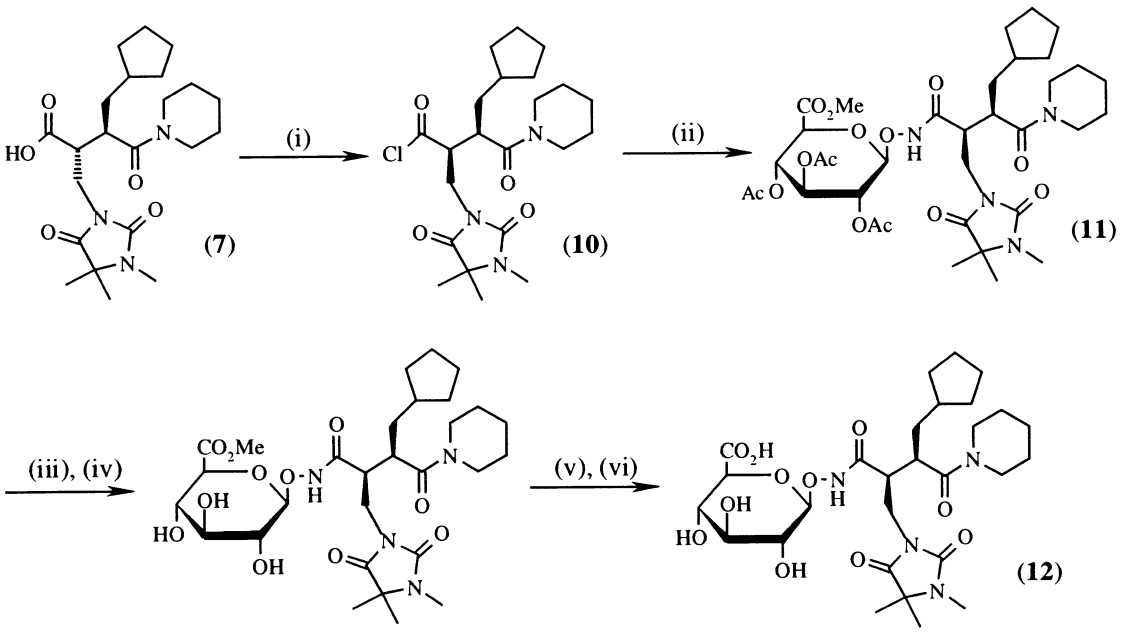
Figure 3.

It was clear that an *N*-protected hydroxylamine would be required for the synthesis of (**6**) since free hydroxylamine is known to react with sugars to afford oximes. The attempted coupling of (**5**) with *N*-hydroxyphthalimide (HONFt) under Mitsunobu conditions returned largely unreacted sugar. However, compound (**8**) was successfully prepared as depicted in Scheme 2, based upon a method previously reported by Roy.[6]

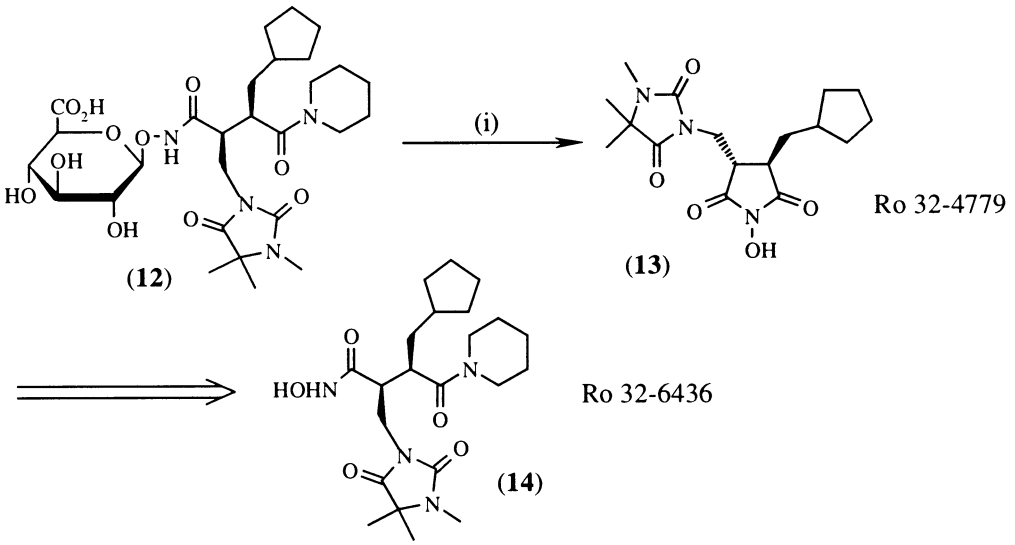


Scheme 2. *Reagents*: (i) NaOMe/MeOH; (ii) acetic anhydride/pyridine (40% over two steps); (iii) HBr/AcOH (82%); (iv) HOFt/TBAHS/$NaHCO_3$/CH_2Cl_2 (66%); (v) hydrazine hydrate (1 equiv.)/MeOH 20 min (quant.)

The starting material 'glucurone' is readily available,[7] and the intermediates are nicely crystalline. The coupled product was obtained exclusively as the β-anomer, and was deprotected to **9** by brief exposure to an equimolar quantity of hydrazine hydrate; the ester groups were unaffected under these conditions.[8] Carboxylic acid (**7**), a key intermediate in the synthesis of Trocade™ was converted to the acid chloride, and successfully coupled to (**9**), albeit in low yield. The product (**11**) appeared to be a single stereoisomer by ^{1}H NMR spectroscopy.



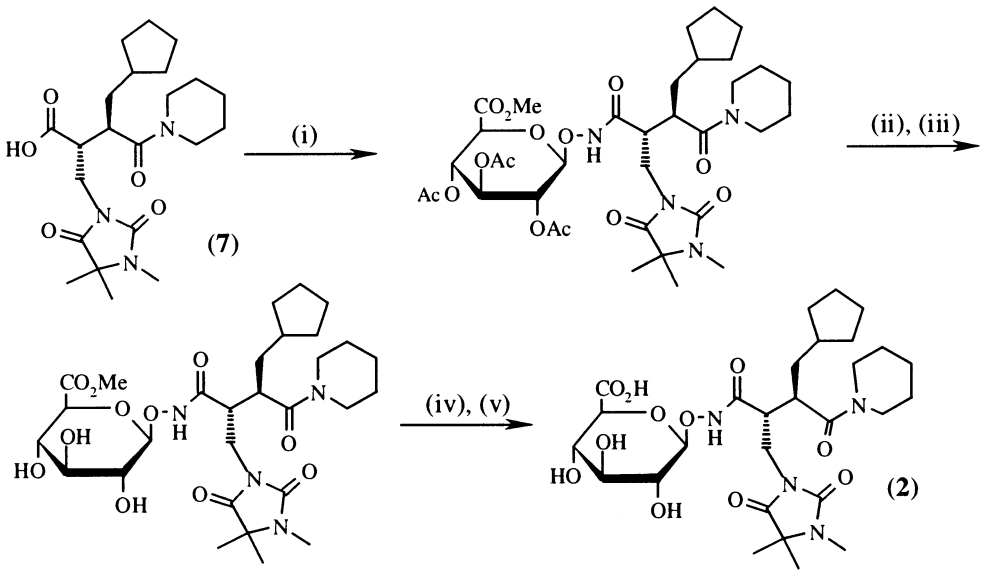
Scheme 3. *Reagents*: (i) $(COCl)_2$/DMF (cat.)/toluene (quant.); (ii) (**9**)/triethylamine/CH_2Cl_2 (24%); (iii) NaOMe (cat.)/MeOH; (iv) Duolite C255(H^+) resin (97%, two steps); (v) 1 M NaOH (aq.); (vi) Duolite C255(H^+) resin followed by lyophilization (97%, two steps)



Scheme 4. *Reagents*: (i) β-glucuronidase/deuterated acetate buffer 40°C

The acetyl groups were rapidly cleaved by treatment with sodium methoxide/methanol and the methyl ester subsequently saponified (Scheme 3). The product (**12**) had the desired m/z but the ^1H NMR was not identical when compared with the authentic metabolite. The material also had a tendency to undergo slow intramolecular cyclization in solution, as judged by LC–MS analysis; this behaviour was not observed for the authentic metabolite. The attempted cleavage

of (**12**) by a β-glucuronidase (in an NMR tube experiment) did not furnish Trocade™, but the cyclic compound (**13**) [Ro 32-4779] which must be derived from the Trocade™ epimer (**14**), Ro 32-6436 (Scheme 4).[9] The epimerisation almost certainly occurred at the acid chloride stage, the low solubility of (**10**) suggesting the origin may be crystallisation induced.[10] This epimerisation has also been observed independently by colleagues at F. Hoffmann-La Roche, Basel.[11]



Scheme 5. *Reagents*: (i) (**9**)/EDAC/HOBT/*N*-ethyl morpholine/CH_2Cl_2; (ii) NaOMe (cat.)/MeOH; (iii) Duolite C255(H^+) resin (92%, two steps); (iv) 1 M NaOH (aq.); (v) Duolite C255(H^+) resin followed by prep. HPLC and lyophilization (60%, two steps)

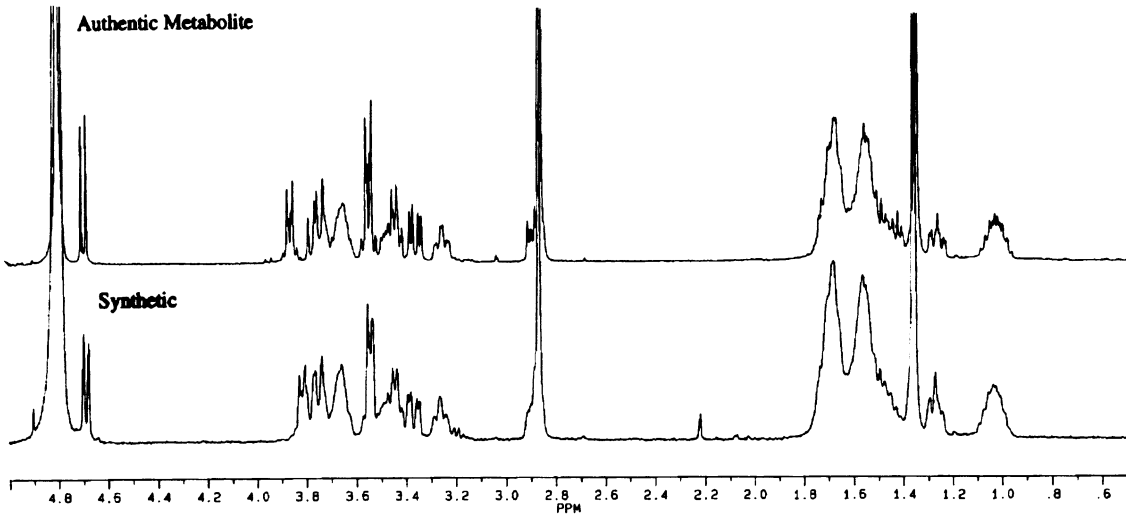


Figure 4. 400 MHz ^1H NMR

The synthesis was now repeated, using an HOBT/EDAC mediated coupling between the sugar (**9**) and acid (**7**). Subsequent steps were performed as before and the final product (**2**) purified by preparative HPLC (Scheme 5). The product was identical (Fig. 4) in all respects to the authentic metabolite (^{1}H NMR, m/z, LC) and did not show any tendency for intramolecular cyclization. This confirmed the metabolite possessed structure (**2**), with the aglycone linked to the sugar through oxygen.

In summary, the structure of the glucuronide adduct of Trocade™ has been shown to be **2** by means of an unambiguous chemical synthesis. A practical route to **9** has been demonstrated, an intermediate which should prove useful for the preparation of glucuronide adducts of various hydroxamic acids.

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