A new class of NO-donor pro-drugs triggered by \(\gamma\)-glutamyl transpeptidase with potential for reno-selective vasodilatation†

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There are a wide range of NO-donor drugs in existence,† including conventional organic nitrates and nitrites, S-nitrosothiols, NONOates and N-hydroxyguanidines (NHGs).12–16 The NHGs are analogues of \(N^\omega\)-hydroxy-\(\omega\)-arginine (NOHA), a biosynthetic intermediate involved in the generation of NO from \(\omega\)-arginine.11 Several enzymatically activated NOG pro-drugs have been reported such as peptidylglycine \(\varepsilon\)-amidating monooxygenase (PAM)-active \(O\)-carboxymethyl \(N\)-hydroxyguanidines17 and \(N\)-\(\beta\)-galactosidases-active (\(\beta\)-\(n\)-galactopyranos-1-\(y\))oxoguanidine.18 Our approach aimed to mask the NO generating NHG group with a \(\gamma\)-glutamyl residue to facilitate activation by the enzyme, \(\gamma\)-glutamyl transpeptidase (\(\gamma\)-GT). Given that \(\gamma\)-GT is primarily expressed in the kidney (5–10 fold higher than in the liver and pancreas),19 it was envisaged that this enzyme could be used to trigger reno-selective release of an NHG and subsequent \textit{in situ} generation of NO (Scheme 1). A similar strategy has been described for reno-selective \(\varepsilon\)-3,4-dihydroxyphenylalanine (\(\varepsilon\)-DOPA), the Glu-DOPA20,21

However, the direct coupling of NHGs with a \(\gamma\)-glutamyl residue was hampered by intramolecular cyclization and dehydration leading to a 1,2,4-oxadiazole ring; or alternatively lactamization and release of a pyroglutamic acid (Scheme 2, data not included).

In an effort to prevent these modes of cyclization, we investigated the use of a bridge between the NHG and the \(\gamma\)-glutamyl group. Both \(\gamma\)-glutamyl itself and \(\gamma\)-aminobutyrol (GABA)22 were explored as linkers. Thus 2a and 2b became synthesis targets (Scheme 3) and they were prepared \textit{via} appropriately protected dipeptide intermediates (ESI;† Scheme S1). Unfortunately 2a gradually decomposed presumably due to the carboxylic acid moieties promoting autodegradation.

\[ \text{Scheme 1} \quad \text{Approach to } \gamma\text{-GT triggered release of NHG 1 and the reno-selective release of nitric oxide.} \]
On the other hand, 2b could be purified by preparative HPLC but was found to be resistant to γ-GT-mediated cleavage in vitro and was considered not to be a useful pro-drug. This prompted the preparation of 3 (Scheme 3), involving the conjugation of only one GABA-Glu dipeptide onto a hydroxamic acid, an alternative NO-donor. Compound 3 too, unfortunately, was found to be resistant to γ-GT mediated decylation, suggesting that the GABA-Glu peptide linker is not suitable for γ-GT cleavage in this setting.

γ-Glutamyl anilines are known substrates for γ-GT\(^{21}\) and presented an alternative linker option. The success of such an approach would involve a 1,6-elimination following the action of γ-GT on \(N^γ\)-glutamylaminobenzyloxy-guanidine 4a–c, as illustrated in Scheme 4. Similar spacers have been employed previously in anticancer pro-drug design.\(^{24}\)

In the event, the synthesis of 4a–c was successfully accomplished through a six-step reaction sequence (Scheme 4). Firstly, γ-glutamylation of 4-aminobenzylalcohol with Alloc-\(\gamma\)-glutamic acid 1-allyl ester (Alloc-Glu-OAll) (ESI† Scheme S1) gave benzyl alcohol 5. Conversion of the benzylalcohol moiety to the corresponding bromide 6 followed by nucleophilic displacement with BocNH\(\text{HCl}\) generated aminoacid 7, and then treatment with \(\text{CF}_3\text{COOH−DCM}\) gave the key intermediate 8 which was coupled with the required amino(alkyl/aryliminio)-methanesulphonate 9a–c to generate 10a–c. Finally the All/Alloc groups were removed under neutral conditions with \([\text{Pd(PPh}_3]_4]/\text{PhSiH}_3\) to give 4a–c.

The same aminobenzyl linker was also used for the γ-glutamylation of \(N\)-hydroxy-formamidines (NHFs) (Scheme 5), \(N^\gamma\)-hydroxy-\(N\)-(4-butyl-2-methylphenyl)formamidine\(^{25}\) and \(N^\gamma\)-hydroxy-\(N\)-(3-chloro-4-morpholino-4-ylphenyl)formamidine\(^{26}\) have been documented as 20-hydroxyicosatetraenoic acid (20-HETE) inhibitors. 20-HETE is a major metabolite of arachidonic acid and is a potent vasoconstrictor; localisation of an NHF would counter the effect of 20-HETE and induce a synergic vasodilation effect mediated by NO. Thus \(N^\gamma\)-hydroxy phenylethylformamidine 12 was prepared in this study and converted to pro-drug 14.

Pro-drugs 4a–c and 14 were rapidly cleaved by γ-GT and they were completely deacylated after 1 h, as judged by LC-MS. Fig. 1(a) and (b) illustrates the LCMS trace of 4b and the conversion of 4b to deacylated intermediate 15 [M-Glu\(^+\)] by γ-GT. This was in clear contrast to the GABA-linked candidates 2b and 3, which proved to be resistant to the action of γ-GT. 1,6-Elimination and loss of the linker from 15 to generate the parent NHG 1b is significantly slower (trace amount of parent 1b was detected by selective ion monitoring at \(m/z\) 180) than the cleavage of the γ-glutamyl moiety. In preliminary experiments with animal tissue, LC-MS analysis revealed ~90% conversion of 4b (100 \(\mu\)M) to 1b in a rat renal homogenate (37 °C; 45 min). In addition, 4b was found to induce substantial vasodilatation in rat isolated perfused kidney preparations (50% of maximum vasodilatation induced by ~40 \(\mu\)M 4b). Details of the bioactivity of these pro-drugs will be reported elsewhere.

In summary, several candidate NO-donor pro-drugs have been prepared, designed for activation by γ-GT. The pro-drugs...
comprise the parent NO-donor, a linker and a γ-glutamyl moiety. GABA-linked pro-drugs are not suitable substrates for \(\gamma\)-GT, but those linked by the aminobenzyl moiety proved to be good substrates for the enzyme. The γ-glutamyl group is cleaved rapidly, with a slower decomposition of the aminobenzyl linker. Improved design is now focussed on tuning the spacer to encourage a more rapid release of the parent NHG drug.

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References


Fig. 1 LCMS trace of 4b incubated in Krebs buffer at 37 °C for 1 h (a) without \(\gamma\)-GT and glutamyl acceptor Gly–gly, 4b is intact; (b) with \(\gamma\)-GT (100 mU mL\(^{-1}\)) and glutamyl acceptor Gly–gly (5 mM), 4b is deglutamylated to give the species 15.