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

Qingzhi Zhang,†a Agnieszka Kulczynska,†a David J. Webb,†b Ian L. Megson*,†c and Nigel P. Botting†*†

There are a wide range of NO-donor drugs in existence,1 including conventional organic nitrates and nitrates, S-nitrosothiols, NONOates and \( N \)-hydroxyguanidines (NHGs).12-16 The NHGs 1 are analogues of \( N \)-\( \alpha \)-hydroxy-\( \gamma \)-arginine (NOHA), a biosynthetic intermediate involved in the generation of NO from \( \gamma \)-arginine.11 Several enzymatically activated NHG pro-drugs have been reported such as peptidylglycine \( \alpha \)-amidating mono-oxygenase (PAM)-active \( O \)-carboxymethyl \( N \)-hydroxyguanidines17 and \( N \)-[\( \beta \)-galactosidas]-active (\( \beta \)-galactopyranos-1-yl)oxyguanidines.18 Our approach aimed to mask the NO generating 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 \( \gamma \)-3,4-dihydroxyphenylalanine (\( \gamma \)-DOPA), the Glu-DOPA.20,21

However, the direct coupling of NHGs with a \( \gamma \)-glutamyl residue was hampered by intramolecular cyclization and dehydration leading to a 1,2,4-oxidiazole 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 \( \gamma \)-aminobutanol (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.

![Scheme 1: Approach to \( \gamma \)-GT triggered release of NHG 1 and the reno-selective release of nitric oxide.](image-url)
γ-Glutamyl anilines are known substrates for γ-GT 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-γ-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 BocNHOH generated aminoxyde 7, and then treatment with CF3COOH–DCM, gave the key intermediate 8 which was coupled with the required amino(alkyl/aryliminio)ethanesulfonate 9a–c to generate 10a–c. Finally the Alloc and Boc groups were removed under neutral conditions with [(Pd(PPh3)4)/PhSiH3] to give 4a–c.

The same aminobenzyl linker was also used for the γ-glutamylation of N-hydroxyformamidines (NHFs) (Scheme 5). N′-Hydroxy-N-(4-butyl-2-methylphenyl)formamidine25 and N′-hydroxy-N-(3-chloro-4-morpholin-4-ylphenyl)formamidine26 have been documented as 20-hydroxyeicosatetraenoic 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′-hydroxyphenylethylformamidine 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 μM) to 1b in a rat renal homogenate (37 °C; 45 min). In addition, 4b was found to induce substantial vasodilation in rat isolated perfused kidney preparations (50% of maximum vasodilatation induced by ~40 μM 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 γ-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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