ChemComm

RSC Publishing

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2013, **49**, 1389

Received 21st November 2012,
Accepted 20th December 2012

DOI: 10.1039/c2cc38382a

www.rsc.org/chemcomm

A new class of NO-donor pro-drugs triggered by γ-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‡*[a]

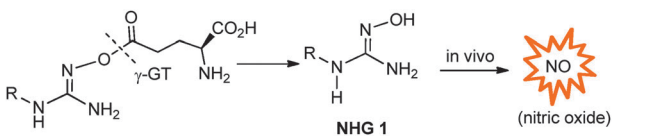
This *communication* describes the synthesis of a new class of *N*-hydroxyguanidine (NHG) pro-drugs which release nitric oxide (NO), triggered by the action of γ-glutamyl transpeptidase (γ-GT), and have potential for the treatment of acute renal injury/failure (ARI/ARF).

Acute renal injury (AKI), or failure (ARF), is a common complication that affects millions of people worldwide, particularly in intensive care units, where it is associated with a mortality rate of between 50% and 80%.[1] There is no effective pharmaceutical therapy to date. One of the major causes of AKI is ischemia-reperfusion injury,[2,3] following aortic ring cross-clamping during by-pass surgery, which can lead to renal ischemia.[4] Reperfusion of ischemic renal tissue causes the generation of reactive oxygen species which induce renal cell injury[5] and promote impairment of renal perfusion at least in part *via* inactivation of the vasodilator, nitric oxide (NO).[6–8] Thus, a kidney selective vasodilator with antioxidant properties is attractive to maintain blood flow to offset AKI and scavenge the reactive oxygen species. Localisation of activity to the kidney would avoid a systemic reduction in blood pressure. Dopamine and fenoldopam, specific agonists of the dopamine-1 receptor, have been used clinically in an effort to reduce the risk of perioperative renal dysfunction, but the effectiveness of these agents is not clear.[9,10] We hypothesised that an effective exogenous NO-donor, which selectively increases renal vasodilatation, would offer an alternative.

There are a wide range of NO-donor drugs in existence,[11] including conventional organic nitrates and nitrites, *S*-nitrothiols, NONOates and *N*-hydroxyguanidines (NHGs).[12–16] The NHGs **1** are analogues of N^{ω}-hydroxy-L-arginine (NOHA), a biosynthetic intermediate involved in the generation of NO from L-arginine.[11] Several enzymatically activated NHG pro-drugs have been reported such as peptidylglycine α-amidating monooxygenase (PAM)-active *O*-carboxymethyl *N*-hydroxyguanidines[17] and *N*-β-galactosidases-active (β-D-galactopyranos-1-yl)oxyguanidine.[18] Our approach aimed to mask the NO generating *N*-OH group with a γ-glutamyl residue to facilitate activation by the enzyme, γ-glutamyl transpeptidase (γ-GT). Given that γ-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 *in situ* generation of NO (Scheme 1). A similar strategy has been described for reno-selective L-3,4-dihydroxyphenylalanine (L-DOPA), the Glu-DOPA.[20,21]

However, the direct coupling of NHGs with a γ-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 γ-glutamyl group. Both γ-glutamyl itself and γ-aminobutanoyl (GABA)[22] were explored as linkers. Thus **2a** and **2b** became synthesis targets (Scheme 3) and they were prepared *via* appropriately protected dipeptide intermediates (ESI;† Scheme S1). Unfortunately **2a** gradually decomposed presumably due to the carboxylic acid moieties promoting autodegradation.

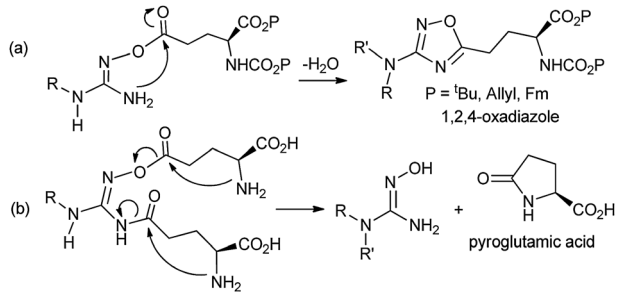


Scheme 1 Approach to γ-GT triggered release of NHG **1** and the reno-selective release of nitric oxide.

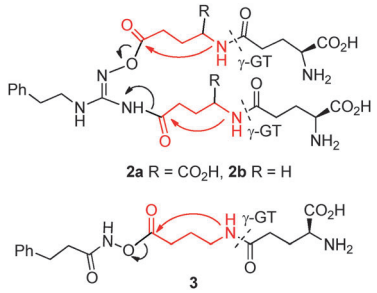
[a] *University of St Andrews, EaStChem School of Chemistry and Centre for Biomolecular Sciences, North Haugh, St Andrews, Fife KY16 9ST, UK. E-mail: qz@st-andrews.ac.uk; Fax: +44 (0)1334 463808; Tel: +44 (0)1334 467274*
[b] *Centre for Cardiovascular Science, The Queen's Medical Research Institute, The University of Edinburgh, 47 Little France Crescent, Edinburgh, EH16 4TJ, UK*
[c] *Free Radical Research Facility, Department of Diabetes and Cardiovascular Science, The University of The Highlands & Islands, Centre for Health Science, Old Perth Road, Inverness, IV2 3JH, UK. E-mail: ian.megson@uhi.ac.uk; Fax: +44 (0)1463 711245; Tel: +44 (0)1463 279562*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2cc38382a

‡ N. P. Botting died on 4th June 2011.

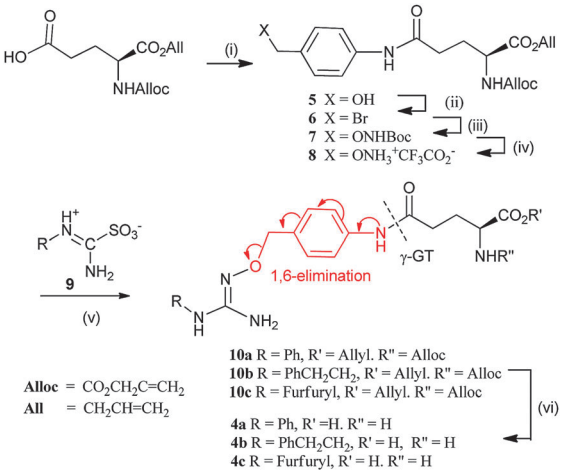


Scheme 2 Cyclization of direct coupling of NHGs with γ-glutamyl residue(s).



Scheme 3 Design of Glu/Gaba linked γ-glutamyl NO-donor pro-drugs of NHG and hydroxamic acid.

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.[11] Compound **3** too, unfortunately, was found to be resistant to γ-GT mediated deacylation, suggesting that the GABA-Glu peptide linker is not suitable for γ-GT cleavage in this setting.



Scheme 4 Design and synthesis of aminobenzyl linked γ-glutamyl NO-donor pro-drugs of NHG: (i) 4-aminobenzylalcohol, EEDQ, DCM, rt, 12 h, 85%; (ii) PBr_3, THF, 0 °C, 2 h, 87%; (iii) BocNHOH, NaH, THF, 0 °C, 4 h, 83%; (iv) CF_3CO_2H, DCM, 92%; (v) **9a** R = Ph or **9b** R = $PhCH_2CH_2$ or **9c** R = furfuryl, Et_3N, DMAP, DCM, 38–53%; (vi) [$Pd(PPh_3)_4$], $PhSiH_3$, DCM, 37–89%.

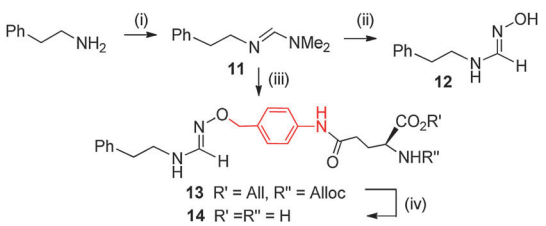
γ-Glutamyl anilines are known substrates for γ-GT[23] and presented an alternative linker option. The success of such an approach would involve a 1,6-elimination following the action of γ-GT on *N*-γ-glutamylaminobenzyloxy-guanidine **4a–c**, as illustrated in Scheme 4. Similar spacers have been employed 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-L-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 aminooxide **7**, and then treatment with CF_3COOH–DCM, gave the key intermediate **8** which was coupled with the required amino(alkyl/aryliminio)-methanesulfonate **9a–c** to generate **10a–c**. Finally the All/Alloc groups were removed under neutral conditions with ([$Pd(PPh_3)_4$]/$PhSiH_3$) 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)formamidine[25] and *N*′-hydroxy-*N*-(3-chloro-4-morpholin-4-ylphenyl)formamide[26] 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** $[M\text{-}Glu]^+$ 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 vasodilatation 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



Scheme 5 Synthesis of *N*-hydroxyformamidine and its glutamyl pro-drug: (i) $Me_2NCH(OMe_2)$, reflux, 2 h, quantitative; (ii) NH_2OHHCl, MeOH, 63%; (iii) **8**, THF, reflux, 29%; (iv) [$Pd(PPh_3)_4$], $PhSiH_3$, DCM, rt, 6 h, 53%.

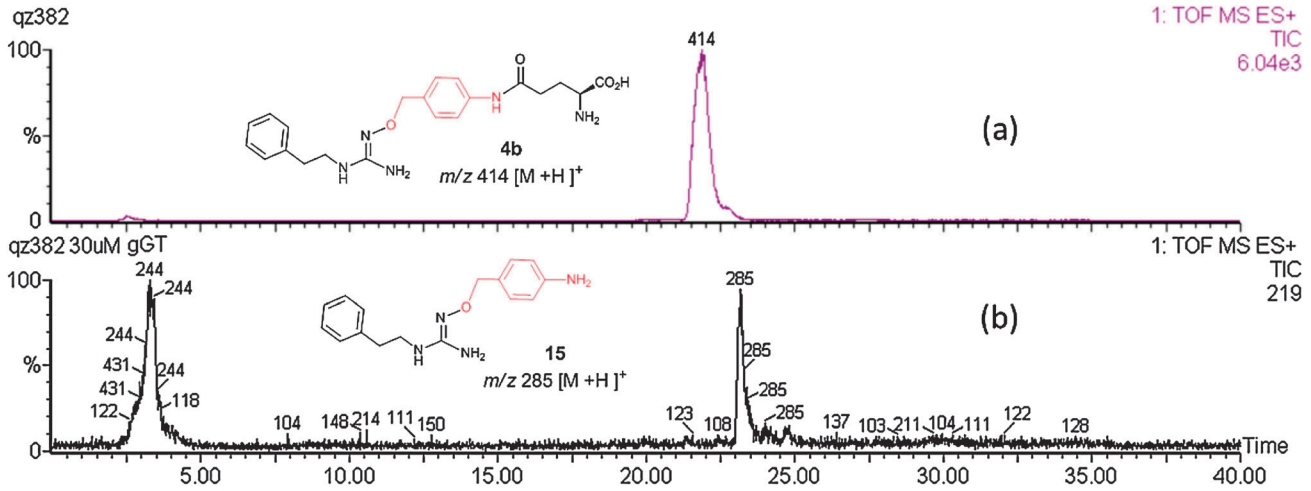


Fig. 1 LCMS trace of **4b** incubated in Krebs buffer at 37 °C for 1 h (a) without γ-GT and glutamyl acceptor Gly–gly, **4b** is intact; (b) with γ-GT (100 mU mL^{-1}) and glutamyl acceptor Gly–gly (5 mM), **4b** is deglutamylated to give the species **15**.

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.

The authors are grateful to the Wellcome Trust (Catalyst Biomedica Development Award 063729/Z/01/Z) for financial support. Thanks go to Prof. David O'Hagan (University of St Andrews) for his input into manuscript preparation.

References

1 R. W. Schrier, W. Wang, B. Poole and A. Mitra, *J. Clin. Invest.*, 2004, **114**, 5–14.
2 J. V. Bonvebtre and J. M. Weinberg, *J. Am. Soc. Nephrol.*, 2003, **14**, 2199–2210.
3 T. A. Sutton, C. J. Fisher and B. A. Molitoris, *Kidney Int.*, 2002, **62**, 1539–1549.
4 G. Mariscalco, R. Lorusso, C. Dominici, A. Renzulli and A. Sala, *Ann. Thorac. Surg.*, 2011, **92**, 1539–1547.
5 P. A. Grace, *Br. J. Surg.*, 1994, **81**, 637–647.
6 M. L. Brocq, S. J. Leslie, P. Milliken and I. L. Megson, *Antioxid. Redox Signaling*, 2008, **10**, 1631–1674.
7 A. MacKenzie and W. Martin, *Br. J. Pharmacol.*, 1998, **124**, 710–728.
8 M. Saran, C. Michel and W. Bors, *Free Radical Res. Commun.*, 1990, **10**, 221–226.
9 M. D. Denton, G. M. Chertow and H. R. Brady, *Kidney Int.*, 1996, **49**, 4–14.
10 T. Bove, G. Landoni, M. G. Calabrò, G. Aletti, G. Marino, E. Cerchierini, G. Crescenzi and A. Zangrillo, *Circulation*, 2005, **111**, 3230–3235.
11 P. G. Wang, T. B. Cai and N. Taniguchi, *Nitric Oxide Donors: For Pharmaceutical and Biological Applications*, Wiley-VCH, 2005, ISBN, 3-527-31015-0.
12 A. Renodon-Cornière, S. Dijols, C. Perollier, D. Lefevre-Groboillot, J. L. Boucher, R. Attias, M. A. Sari, D. Stuehr and D. Mansuy, *J. Med. Chem.*, 2002, **45**, 944–954.
13 D. Mansuy and J. L. Boucher, *Drug Metab. Rev.*, 2002, **34**, 593–606.
14 M. Xian, X. P. Li, X. P. Tang, X. C. Chen, X. L. Zheng, J. J. Galligan, D. L. Kreulen and P. G. Wang, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2377–2380.
15 M. Xian, N. Fujiwara, T. Cai, Z. Wen, S. Kazuma, A. Janczuk, X. Tang, V. Telyatnikov, Y. Miyamoto, N. Taniguchi and P. G. Wang, *Bioorg. Med. Chem.*, 2002, **10**, 3049–3055.
16 Q. Jia, T. Cai, M. Huang, H. Li, M. Xian, T. L. Poulos and P. G. Wang, *J. Med. Chem.*, 2003, **46**, 2271–2274.
17 D. Schade, J. Kotthaus, H. Hungeling, J. Kotthaus and B. Clement, *ChemMedChem*, 2009, **4**, 1595–1599.
18 D. Schade, J. Kotthaus, N. Klein, J. Kotthaus and B. Clement, *Org. Biomol. Chem.*, 2011, **9**, 5249–5259.
19 J. P. Ward, *Ann. R. Coll. Surg. Engl.*, 1975, **57**, 248–261, Academic Press.
20 S. Wilk, H. Mizoguchi and M. Orlowski, *J. Pharmacol. Exp. Ther.*, 1978, **206**, 227–232.
21 M. Barthelmebs, A. Caillette, J. D. Ehrhardt, J. Velly and J. L. Imbs, *Kidney Int.*, 1990, **37**, 1414–1422.
22 R. G. G. Leenders, K. A. A. Gerrits, R. Ruijtenbeek and H. W. Scheeren, *Tetrahedron Lett.*, 1995, **36**, 1701–1704.
23 A. Ménard, R. Castonguay, C. Lherbet, C. Rivard, Y. Roupioz and J. W. Keillor, *Biochemistry*, 2001, **40**, 12678–12685.
24 B. E. Toki, C. G. Cerveny, A. F. Wahl and P. D. Senter, *J. Org. Chem.*, 2002, **67**, 1866–1872.
25 M. Sato, T. Ishii, Y. Kobayashi-Matsunaga, H. Amada, K. Taniguchi, N. Miyata and K. Kameo, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2993–2995.
26 N. Miyata, T. Seki, Y. Tanaka, T. Omura, K. Taniguchi, M. Doi, K. Bandou, S. Kametani, M. Sato, S. Okuyama, L. Cambj-Sapunar, D. R. Harder and R. J. Roman, *J. Pharmacol. Exp. Ther.*, 2005, **314**, 77–85.