ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 2083–2085

www.rsc.org/chemcomm

COMMUNICATION

Second generation specific-enzyme-activated rotaxane propeptides†

Antony Fernandes,‡[ab] Aurélien Viterisi,‡[a] Vincent Aucagne,*[c] David A. Leigh*[a] and Sébastien Papot*[b]

Received 29th November 2011, Accepted 14th December 2011
DOI: 10.1039/c2cc17458h

A [2]rotaxane, in which the peptidic axle is protected from degradation by the macrocyclic sheath and terminated with a novel glycosidase-cleavable stopper, is rendered water-soluble by derivatisation with tetra(ethylene glycol) (TetEG) or glucosylated tetra(ethylene glycol) (Glc-TetEG) chains using the CuAAC 'click' reaction. The Glc-TetEG-derivatised rotaxane propeptide is > 50 000 times more soluble in aqueous media than the parent rotaxane. Activation of the water-soluble rotaxane propeptide with a β-galactosidase efficiently releases the parent peptide.

The design of enzyme-responsive rotaxanes is an emerging area of activity with potential biological applications.[1–5] Biodegradable polyrotaxanes have been studied as selective drug and gene delivery systems[1] and enzymatically-triggered 'nanovalves' have been demonstrated to regulate the release of guest molecules stored in mesoporous silica.[2] Recently, we prepared a [2]rotaxane propeptide that protects a pentapeptide thread from degradation from general peptidases whilst allowing the release of the free peptide in a controlled fashion through treatment with a specific glycosidase.[3] Since many bioactive peptides suffer from *in vivo* instability and poor bioavailability, this approach offers some promise for peptide-based therapies. However, the first generation rotaxane propeptide exhibited poor aqueous solubility and had no convenient functional group 'handles' through which the rotaxane could be subsequently derivatised to improve its properties. Here we report on a second generation rotaxane propeptide, **1**, composed of a galactosyl trigger, a novel self-immolative stopper[6] and a triglycyl peptide thread encapsulated by a macrocycle[7] bearing two azide groups (Schemes 1 and 2). The azide groups are suitable for introducing hydrophilic (or other) moieties after rotaxane formation *via* copper(I)-catalysed azide-alkyne 1,3-cycloaddition (CuAAC) 'click' reactions (*e.g.* **2** and **3**).[8]

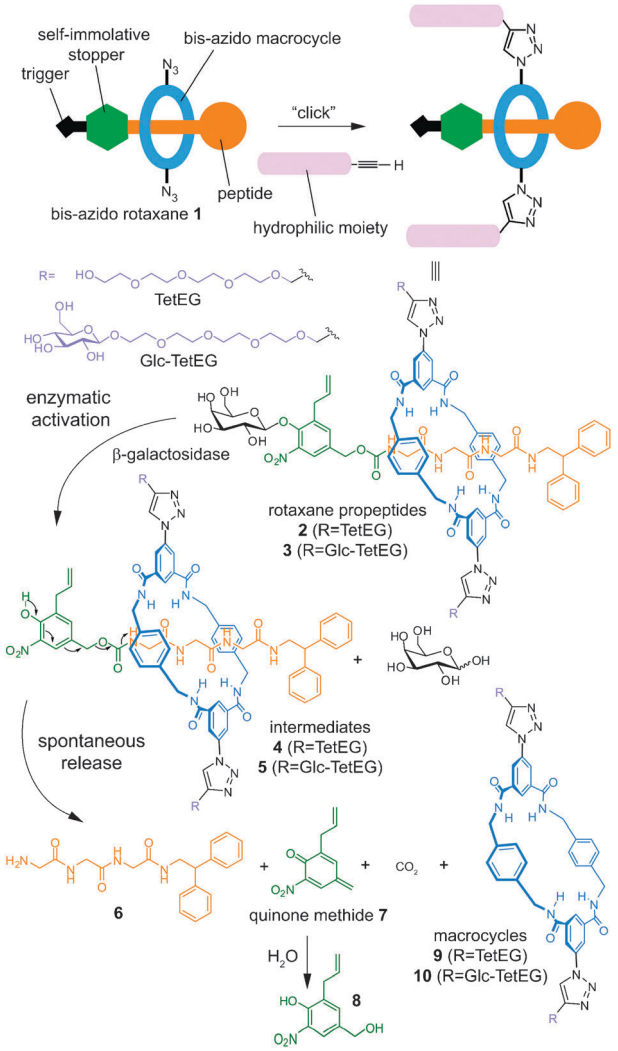
[a] *School of Chemistry, University of Edinburgh, The King's Buildings, West Mains Road, Edinburgh, EH9 3JJ, UK. E-mail: david.leigh@ed.ac.uk; Web: http://www.catenane.net; Fax: (+44) 131-650-6453*
[b] *Université de Poitiers, UMR-CNRS 6514, Laboratoire de Synthèse et Réactivité des Substances Naturelles, 4 rue Michel Brunet, BP 633, 86022 Poitiers, France. E-mail: sebastien.papot@univ-poitiers.fr*
[c] *Centre de Biophysique Moléculaire, UPR 4301 CNRS, Rue Charles Sadron, 45071 Orléans, Cedex 2, France. E-mail: aucagne@cnrs-orleans.fr*
† Electronic supplementary information (ESI) available: Experimental procedures, full characterisation data. See DOI: 10.1039/c2cc17458h
‡ These authors contributed equally to this work.

While the first generation rotaxane propeptide system[3] was limited to peptide axles with bulky N-terminal residues,[9] here we demonstrate that a *bis-meta*-substituted *para*-hydroxybenzyl carbamate stopper can be used as a rotaxane stopper for peptides with an N-terminal amino acid as small as glycine. The proposed release mechanism of propeptides **2** and **3** in the presence of the activating enzyme is shown in Scheme 1. Hydrolysis of the glycosidic bond by β-galactosidase triggers a sequence of reactions that decompose the stopper, and thus the rotaxane architecture, releasing the free peptide **6**.

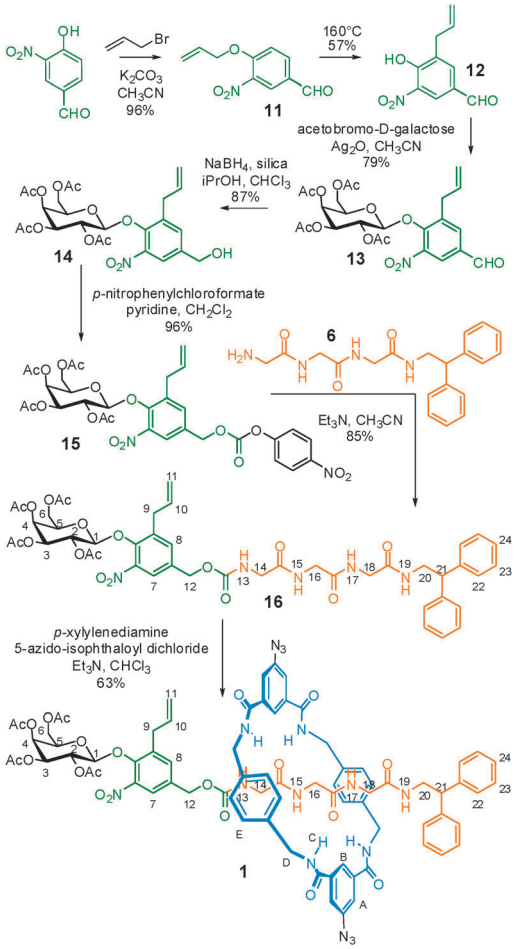
The synthesis of rotaxane **1** was carried out in seven steps from commercially available 4-hydroxy-3-nitrobenzaldehyde (Scheme 2). Alkylation with allyl bromide (K_2CO_3, MeCN, 96%) afforded ether **11**, which underwent a Claisen rearrangement at 160 °C to produce the *bis-ortho*-substituted phenol **12** in 57% yield. Stereoselective glycosylation of **12** with acetobromo-D-galactose (Ag_2O, MeCN, 79%) gave β-galactoside **13**. Reduction of the aldehyde and subsequent treatment with *p*-nitrophenylchloroformate afforded the activated carbonate **15** (96%), which was then coupled with tripeptide **6** to furnish the propeptide thread **16** (85%). Treatment of thread **16** with *p*-xylylenediamine, 5-azido-isophthaloyl dichloride and Et_3N in $CHCl_3$ gave the corresponding glyco[2]rotaxane[3,10] **1** in 63% yield.[11] Rotaxane **1** appears to be indefinitely stable in solution, indicating that the second generation self-immolative stopper is sufficiently bulky to avoid dethreading even with an N-terminal glycine residue on the axle.

Comparison of the ^{1}H NMR spectra of **1** and **16** confirmed the interlocked structure of **1** (Fig. 1). Several of the resonances of the stoppered tripeptide (H_{14}, H_{16}, H_{18}, H_{20} and H_{21}) are significantly shifted to higher field in the rotaxane as a result of the shielding effect of the aromatic rings of the macrocycle. The large shifts (0.4–0.8 ppm) of glycine protons H_{14}, H_{16} and H_{18} indicate that the glycyl residues are located in the cavity of the macrocycle in the predominant co-conformations of **1** in $CDCl_3$. Higher field shifts of the self-immolative stopper protons H_7, H_8 and H_{12} also occur while no changes are observed for the carbohydrate protons (H_{1-6}). This suggests that the macrocycle is able to shuttle along the peptide axle up to the self-immolative stopper unit but not onto the carbohydrate residue.

Bis-azido-glyco[2]rotaxane **1** was then functionalised with TetEG and Glc(Ac)$_4$-TetEG alkynes **17** and **18** using the CuAAC reaction (Scheme 3).[12] The carbohydrate units of rotaxanes **19** and **20** were subsequently deprotected (MeONa, MeOH) to afford propeptides **2** and **3**, respectively (Scheme 3).



Scheme 1 β-Galactosidase-triggered release of triglycyl peptide **6** from the functionalised [2]rotaxane propeptides **2** and **3**.



Scheme 2 Synthesis of *bis*-azido-glyco[2]rotaxane **1**.

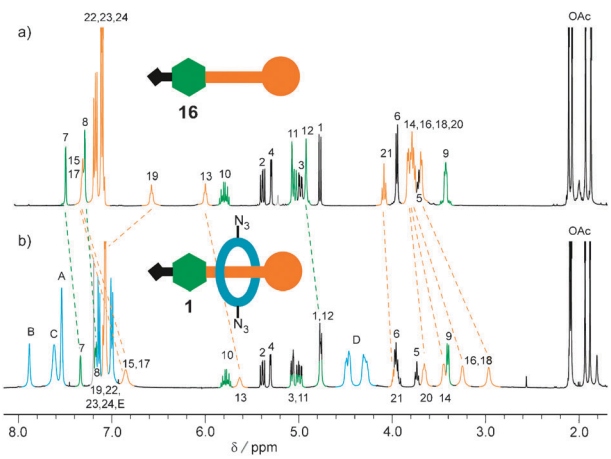


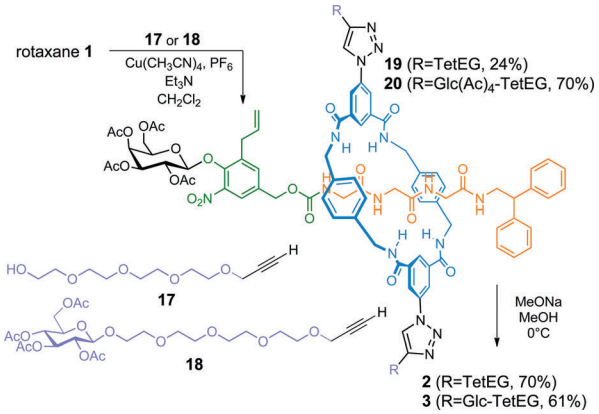
Fig. 1 ^{1}H NMR spectra (400 MHz, 298 K, $CDCl_3$) of (a) thread **16** and (b) [2]rotaxane **1**. The assignments and colours of the signals correspond to that shown in Scheme 2. Residual solvent peaks are shown in gray.

To assess the effect of the hydrophilic side chains on the properties of rotaxanes **2** and **3**, aqueous solubility measurements were carried out. Propeptide thread **21** and rotaxane **22** were synthesised as references for these experiments (Scheme 4). Rotaxane **22** proved to be almost insoluble in water (3.8×10^{-7} mol L^{-1}) whilst propeptide thread **21** was modestly soluble (8.6×10^{-4} mol L^{-1}). Derivatisation of **1** in the form of rotaxane **2** bearing two TetEG substituents gave a 15-fold increase in aqueous solubility (6.0×10^{-6} mol L^{-1}). Introduction of the Glc-TetEG side chains (rotaxane **3**) afforded excellent aqueous solubility of 2.2×10^{-2} mol L^{-1}; 50 000× more soluble than the model rotaxane (**22**) and ~25× higher than that of the parent thread (**21**).

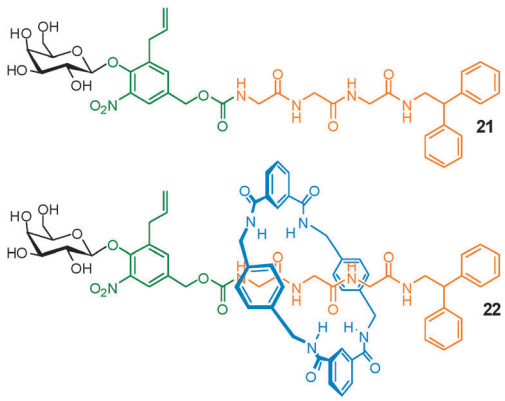
In order to confirm that the water soluble rotaxane **3** can operate as a triggered-release propeptide system, enzymatic activation of **3** was carried out with *E. coli* β-galactosidase in phosphate buffer (0.02 M, pH 7.0) at 37 °C and monitored by HPLC (Fig. 2).

Under these conditions, rotaxane **3** was degraded over 10 h, the disappearance of **3** being accompanied by the appearance of peptide **6** and macrocycle **10** in the HPLC trace (Fig. 2b and c). The benzylic alcohol **8**, formed by hydrolysis of the quinone methide intermediate **7** (Scheme 1), was also detected in the reaction mixture (Fig. 2b and c). The results are consistent with the enzyme-catalysed disassembly of rotaxane **3** proceeding through the self-immolative mechanism shown in Scheme 1.

In summary, we have demonstrated that the aqueous solubility of a rotaxane propeptide can be greatly improved (>50 000×) *via* post-functionalisation of a *bis*-azido-glyco[2]rotaxane using



Scheme 3 CuAAC post-functionalisation of rotaxane **1**.



Scheme 4 Propeptide thread **21** and rotaxane **22**.

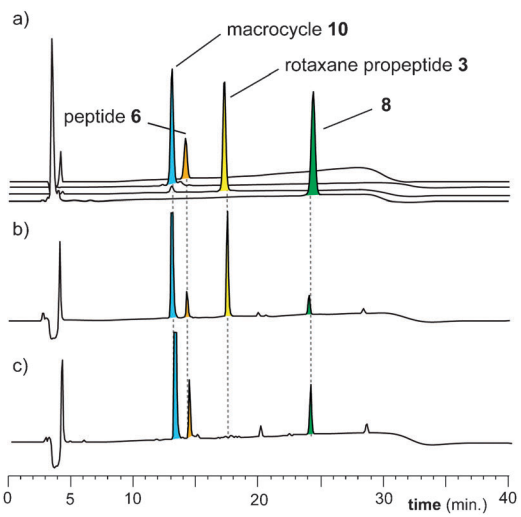


Fig. 2 (a) Superimposed HPLC traces (λ = 220 nm, reverse phase C-18 column) of individual components **3**, **6**, **8** and **10**. (b) and (c) HPLC traces of the enzymatic hydrolysis of rotaxane propeptide **3** with *E. coli* β-galactosidase in phosphate buffer (0.02 M, pH 7.0) at 37 °C using 1000 U/μmol of substrate after (b) 2 h and (c) 10 h (Scheme 1).

the CuAAC reaction. Both the novel self-immolative stopper and the *bis*-azido rotaxane ring employed in this system may prove useful for accessing a wide range of propeptides decorated for specific biological applications.

This work was supported by the Science and Technology Department of the French Embassy in the United Kingdom, the Scottish Executive, the Royal Society of Edinburgh and the Engineering and Physical Sciences Research Council (EPSRC).

Notes and references

1 T. Ooya and N. Yui, *J. Controlled Release*, 1999, **58**, 251; T. Ooya, A. Yamashita, M. Kurisawa, Y. Sugaya, A. Maruyama and N. Yui, *Sci. Technol. Adv. Mater.*, 2004, **5**, 363; C. Moon, Y. M. Kwon, W. K. Lee, Y. J. Park and V. C. Yang, *J. Controlled Release*, 2007, **124**, 43; T. Ooya, H. S. Choi, A. Yamashita, N. Yui, Y. Sugaya, A. Kano, A. Maruyama, H. Akita, R. Ito, K. Kogure and H. Harashima, *J. Am. Chem. Soc.*, 2006, **128**, 3852; X. Zhang, X. Zhu, F. Ke, L. Ye, E. Chen, A. Zhang and Z. Feng, *Polymer*, 2009, **50**, 4343.

2 F. Porta, G. E. M. Lamers, J. I. Zink and A. Kros, *Phys. Chem. Chem. Phys.*, 2011, **13**, 9982; K. Patel, S. Angelos, W. R. Dichtel, A. Coskun, Y.-W. Yand, J. I. Zink and J. F. Stoddart, *J. Am. Chem. Soc.*, 2008, **130**, 2382.

3 A. Fernandes, A. Viterisi, F. Coutrot, S. Potok, D. A. Leigh, V. Aucagne and S. Papot, *Angew. Chem., Int. Ed.*, 2009, **48**, 6443.

4 A stilbene-based rotaxane has been used to control the reactivity of a dipeptidic thread against protease hydrolysis: A. G. Cheetham, M. G. Hutchings, T. D. W. Claridge and H. L. Anderson, *Angew. Chem., Int. Ed.*, 2006, **45**, 1596.

5 For other peptide-derivatised rotaxanes see: V. Dvornikovs, B. E. House, M. Kaetzel, J. R. Dedman and D. B. Smithrud, *J. Am. Chem. Soc.*, 2003, **125**, 8290; V. Aucagne, D. A. Leigh, J. S. Lock and A. R. Thomson, *J. Am. Chem. Soc.*, 2006, **128**, 1784; X. Bao, I. Isaacsohn, A. F. Drew and D. B. Smithrud, *J. Am. Chem. Soc.*, 2006, **128**, 12229; J. Zhu, B. E. House, E. Fleck, I. Isaacsohn, A. F. Drew and D. B. Smithrud, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5058; X. Wang, X. Bao, M. McFarland-Mancini, I. Isaacsohn, A. F. Drew and D. B. Smithrud, *J. Am. Chem. Soc.*, 2007, **129**, 7284; J. Zhu, M. McFarland-Mancini, A. F. Drew and D. B. Smithrud, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 520.

6 S. Papot, I. Tranoy, F. Tillequin, J.-C. Florent and J.-P. Gesson, *Curr. Med. Chem.: Anti-Cancer Agents*, 2002, **2**, 155; I. Tranoy-Opalinski, A. Fernandes, M. Thomas, J.-P. Gesson and S. Papot, *Anti-Cancer Agents Med. Chem.*, 2008, **8**, 618.

7 D. González Cabrera, B. D. Koivisto and D. A. Leigh, *Chem. Commun.*, 2007, 4218; K. D. Hänni and D. A. Leigh, *Chem. Soc. Rev.*, 2010, **39**, 1240.

8 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596; C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057.

9 In the first generation system (ref. 3) a bulky N-terminal amino acid (tyrosine) formed part of the non-glycosylated stopper.

10 F. Coutrot, E. Busseron and J.-L. Montero, *Org. Lett.*, 2008, **10**, 753; F. Coutrot, C. Romuald and E. Busseron, *Org. Lett.*, 2008, **10**, 3741; F. Coutrot and E. Busseron, *Chem.–Eur. J.*, 2008, **14**, 4784; F. Coutrot and E. Busseron, *Chem.–Eur. J.*, 2009, **15**, 5186; E. Busseron, C. Romuald and F. Coutrot, *Chem.–Eur. J.*, 2010, **16**, 10062.

11 D. A. Leigh, A. Murphy, J. P. Smart and A. M. Z. Slawin, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 728; G. W. H. Wurpel, A. M. Brouwer, I. H. M. van Stokkum, A. Farran and D. A. Leigh, *J. Am. Chem. Soc.*, 2001, **123**, 11327; G. Brancato, F. Coutrot, D. A. Leigh, A. Murphy, J. K. Y. Wong and F. Zerbetto, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 4967; M. Asakawa, G. Brancato, M. Fanti, D. A. Leigh, T. Shimizu, A. M. Z. Slawin, J. K. Y. Wong, F. Zerbetto and S. Zhang, *J. Am. Chem. Soc.*, 2002, **124**, 2939; T. Da Ros, D. M. Guldi, A. Farran Morales, D. A. Leigh, M. Prato and R. Turco, *Org. Lett.*, 2003, **5**, 689; G. Bottari, D. A. Leigh and E. M. Pérez, *J. Am. Chem. Soc.*, 2003, **125**, 13360; A. Altieri, V. Aucagne, R. Carrillo, G. J. Clarkson, D. M. D'Souza, J. A. Dunnett, D. A. Leigh and K. M. Mullen, *Chem. Sci.*, 2011, **2**, 1922; R. Ahmed, A. Altieri, D. M. D'Souza, D. A. Leigh, K. M. Mullen, M. Papmeyer, A. M. Z. Slawin, J. K. Y. Wong and J. D. Woollins, *J. Am. Chem. Soc.*, 2011, **133**, 12304.

12 The modest yield (24%) of **19** was largely the result of a challenging purification procedure that required preparative reverse phase HPLC to obtain the *bis*-TetEG-functionalised rotaxane in good purity.