Pergamon

Tetrahedron Letters 42 (2001) 515–518

TETRAHEDRON LETTERS

Solid phase synthesis of trypanothione reductase inhibitors—towards single bead screening

David Orain and Mark Bradley*

Department of Chemistry, University of Southampton, Southampton SO17 1BJ, UK

Received 16 October 2000; accepted 8 November 2000

Abstract—Using solid phase chemistry, inhibitors of trypanothione reductase were synthesised on TentaGel resin using a biologically compatible safety-catch linker. The material released was of high purity, while cleavage kinetics indicated the potential of the system for multiple release.

Over the last decade, solid phase synthesis and combinatorial chemistry have undergone major evolution.[1] In the early years the process of split and mix revolutionised the concept of library synthesis.[2] This strategy is less widely used now, due in large measure to the fact that compound mixtures appeared to be problematic in terms of screening. The alternative approach of single bead screening suffers from many practical and synthetic problems, not the least of which are the vast numbers of screens necessary when screening large split and mix libraries, and compound quantity.

Thus, to apply split and mix methodology effectively, efficient screening processes must be developed which allow the direct identification of the active bead and thus the active compound.[3] Where release is required, it would be ideal if the cleavage could take place directly in a biologically compatible environment. However, despite a wide range of linkers[4] being available, only a few of them are 'biological' compatible. Another important criterion is the purity of the released material because the substance will be screened directly without purification.

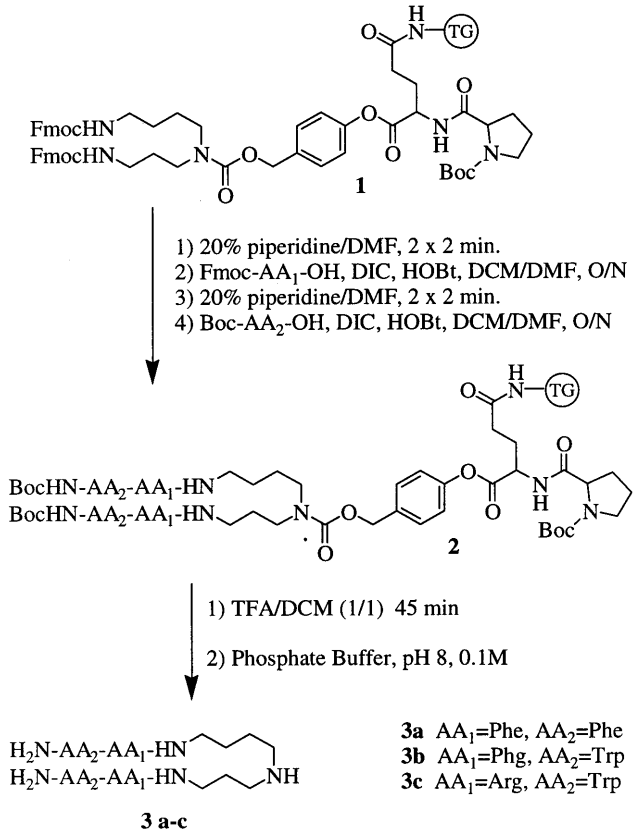
Our group has long been involved in the synthesis and evaluation of trypanothione reductase inhibitors[5] and some previous work has permitted us to discover a 100 nM inhibitor based on a spermidine core. With this knowledge, our goal was to develop a very efficient screening method to identify quickly the active inhibitors from a combinatorial approach by split and mix synthesis. Our choice was based on single bead screening both by zone gel and single well screening methods. Here we wish to report the synthesis of inhibitors of the trypanothione reductase using a pH cleavable linker containing a spermidine scaffold,[6] and discuss the synthesis, kinetics of the cleavage and purities of the cleaved materials.

Starting from the resin **1**,[6] the general strategy was to couple two amino acids, followed by safety-catch linker activation and release of the desired compound under biologically compatible conditions in a buffered solution at pH 8.0. The first step of the synthesis, an Fmoc deprotection, failed under normal conditions (two treatments of 20 minutes with a solution of 20% piperidine in DMF), due to cleavage of the phenyl ester. To overcome this problem, two treatments of only 2 minutes with 20% piperidine in DMF were sufficient to completely remove the Fmoc group without degradation. An Fmoc amino acid was then coupled, with the completion of the coupling being followed by a qualitative ninhydrin test and its efficiency measured by a quantitative Fmoc test. In all the cases, the Fmoc test gave the expected value. A second Fmoc deprotection was then carried out, and a Boc amino acid was coupled to afford the immobilised inhibitor in its Boc protected form. The acid labile protection was removed with 50% of TFA in DCM for 45 minutes also activating the safety-catch linker. After washing with DCM,

Keywords: resin; solid phase synthesis; trypanothione reductase.

* Corresponding author. Tel.: +44 2380 593598; fax: +44 2380 596766; e-mail: mb14@soton.ac.uk

0040-4039/01/$ - see front matter
PII: S0040-4039(00)02009-8



Scheme 1.

DMF, DCM, MeOH and H_2O, the resin was suspended in a phosphate buffer (0.1 M, pH 8.0) to allow the release of the inhibitors (Scheme 1).

The rate of release of each compound **3a**–**c** with time by measuring the intensity of the corresponding peak by HPLC is shown in Fig. 1. The more hydrophobic compounds were released more slowly than the hydrophilic ones. Maximal release was obtained after 2 h for **3c** while for **3b** and **3a**, release was slower (3 and 4 hours, respectively). No degradation of the released

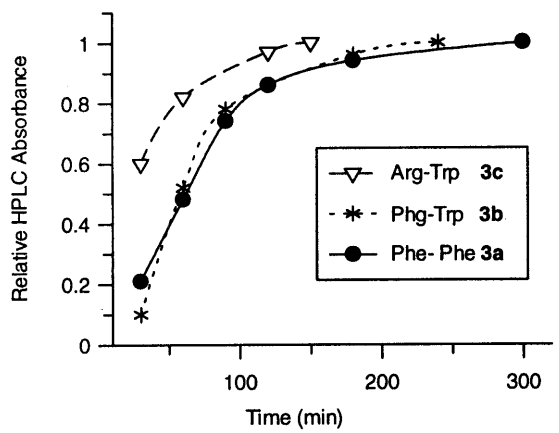


Figure 1. Release of inhibitors with time.

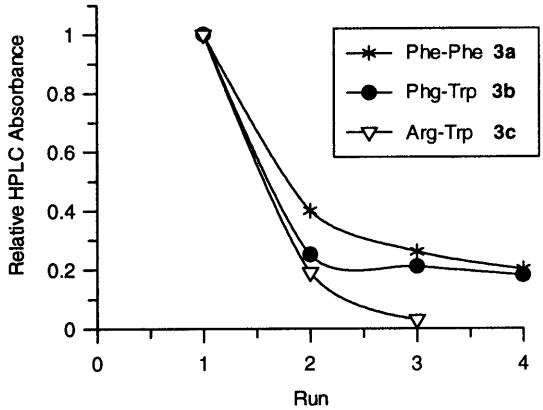


Figure 2. Release of inhibitors with run.

substrates was observed over the longer cleavage times. However, the release was not complete after treatment with the buffer. If the solution was filtered off and the resin resubmitted to fresh buffer for 4 hours, HPLC indicated that more compound was released from the resin, although in the first reaction cleavage had essentially stopped, as indicated by HPLC, while the pH of the solution was still basic, clearly indicating sites within the bead were not accessible or swollen in the first cleavage. This experiment was repeated. The relative amounts of compounds varied in relation to their structure. Thus, hydrophobic compounds were still cleaved even after the fourth treatment, although in much reduced amounts. In each case, the first release was the most important with typically 50–60% cleavage (Fig. 2).

Compounds **3a**, **3b** and **3c** were obtained with purities of 80, 85 and 60%, respectively, by HPLC, with the arginine compound containing some of the mono Pmc protected compound.[7] The HPLC traces are shown in Fig. 3.

These three compounds were purified by semi-preparative HPLC (43, 54 and 30% isolated yields for **3a**, **3b** and **3c**) and their structures confirmed by NMR.

In conclusion, we have demonstrated that trypanothione reductase inhibitors can be obtained with a very good purity following safety-catch linker activation. Moreover, the kinetics of the cleavage showed that multiple release could be achieved and so could lead to the structure of the active inhibitor without the need of an extra encoding method.[3b] Currently, we are investigating enzymatic screening of these inhibitors and direct one-bead/one-compound screening methodologies.

Acknowledgements

M. Bradley thanks the BBSRC (51/BO9512) for funding D. Orain.

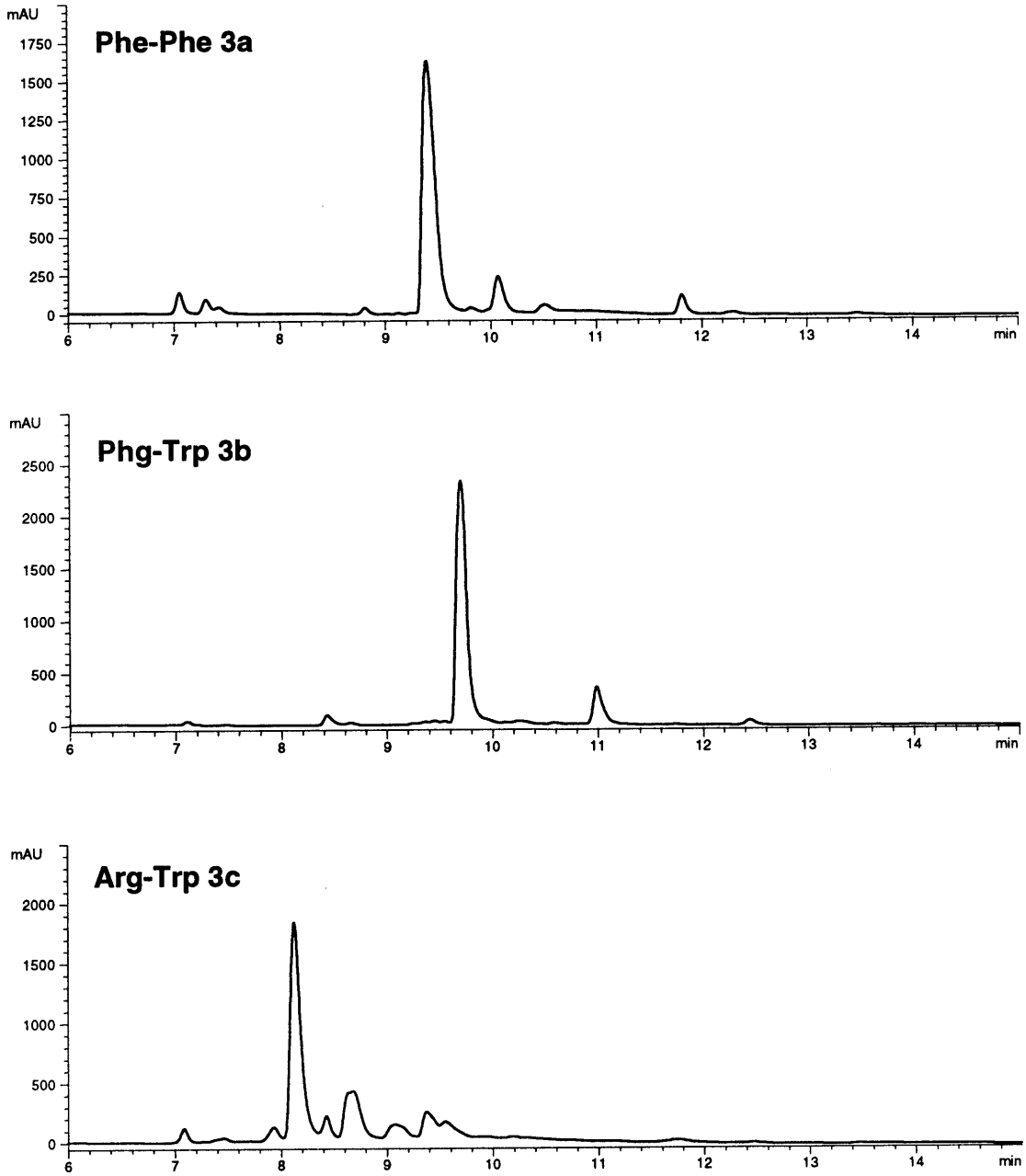


Figure 3. HPLC traces of **3a**, **3b**, **3c** recorded at 220 nm.

References

1. For reviews, see: (a) Terret, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1994**, *51*, 8135. (b) Gordon, E. M.; Gallop, M. A.; Patel, D. V. *Acc. Chem. Res.* **1996**, *29*, 144; (c) Lam, K. S.; Lebl, M.; Krchnák, V. *Chem. Rev.* **1997**, *97*, 411.
2. (a) Furka, A.; Sebeysten, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Prot. Res.* **1991**, *37*, 487. (b) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo J. H. *Nature (London)* **1991**, *354*, 84. (c) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature (London)* **1991**, *354*, 82.
3. (a) Still, W. C. *Acc. Chem. Res.* **1996**, *29*, 155. (b) Meldal, M.; Svendsen, I.; Breddam, K.; Auzanneau, F.-I. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3314. (c) Youngquist, R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *J. Am. Chem. Soc.* **1995**, *117*, 3900. (d) Burbaum, J. J.; Ohlmeyer, M. H.; Reader, J. C.; Henderson, I.; Dillard, L. W.; Li, G.; Randle, T. L.; Signal, N. H.; Chelsky, D.; Baldwin, J. J. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 6027. (e) Baldwin, J. J.; Burbaum, J. J.; Henderson, I.; Ohlmeyer, M. H. *J. Am. Chem. Soc.* **1995**, *117*, 5588. (f) Salmon, S. E.; Lam, K. S.; Lebl, M.; Kandola, A.; Khattri, P. S.; Wade, S.; Patek, M.; Kocis, P.; Krchnak, V.; Thorpe, D.; Felder, S. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11708.

4. (a) James, I. W. *Tetrahedron* **1999**, *55*, 4855. (b) Guillier, F.; Orain, D.; Bradley, M. *Chem. Rev.* **2000**, 2091.
5. (a) Marsh, I. R.; Smith, H. K.; Bradley, M. *Chem. Commun.* **1995**, 941. (b) Marsh, I. R.; Smith, H. R.; Leblanc, C., Bradley, M. *Mol. Div.* **1996**, *2*, 165. (c) Marsh, I. R.; Bradley, M. *Eur. J. Biochem.* **1997**, *243*, 690 (d) Marsh, I. R.; Bradley, M. *Tetrahedron* **1997**, *53*, 17317. (e) Smith, H. R.; Bradley, M. *J. Comb. Chem.* **1999**, *1*, 326.
6. Orain, D.; Bradley, M. *Mol. Div.*, in press.
7. Smith, H. K. Ph.D. Thesis, University of Southampton, 1998.