Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 6999–7001
Tetrahedron Letters

A novel approach to the regioselective synthesis of a disulfide-linked heterodimeric bicyclic peptide mimetic of brain-derived neurotrophic factor

Jordan M. Fletcher and Richard A. Hughes*

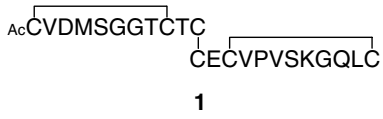
Department of Pharmacology, The University of Melbourne, Victoria 3010, Australia

Received 22 June 2004; revised 19 July 2004; accepted 2 August 2004

Abstract—We describe a novel acetamidomethyl to *S*-pyridinyl exchange that is used for the synthesis of a multi-disulfide-linked and constrained heterodimeric bicyclic peptide mimetic of brain-derived neurotrophic factor (BDNF). This simple and effective method should be readily transferable to the synthesis of similar disulfide-linked heterodimeric peptides, as well as being of general utility for the synthesis of peptides bearing multiple cystine frameworks.

Methods for the formation of unsymmetrical disulfide bonds are required for the synthesis of many biologically active peptides and peptide mimetics. There is a range of *S*-protecting groups—and strategies for their removal—that offer sufficient orthogonality to enable the selective synthesis of compounds with four or more disulfide bonds. Nevertheless, the caveats associated with the use and manipulation of these protecting groups continue to drive the search for alternative *S*-protection strategies. In this letter, we introduce a simple technique for the conversion of an acetamidomethyl (Acm) function to the corresponding *S*-pyridinyl (SPy) adduct. We subsequently apply this method to the synthesis of a heterodimeric bicyclic peptide mimetic of brain-derived neurotrophic factor (BDNF).

Our laboratory has previously described novel cyclic peptides as mimetics of BDNF, including monomeric monocyclic antagonists,[1] homodimeric bi- and tricyclic agonists,[2] and more recently, heterodimeric bicyclic compounds. One of these latter compounds **1** (Fig. 1) proved to be a particularly challenging synthetic target.



1

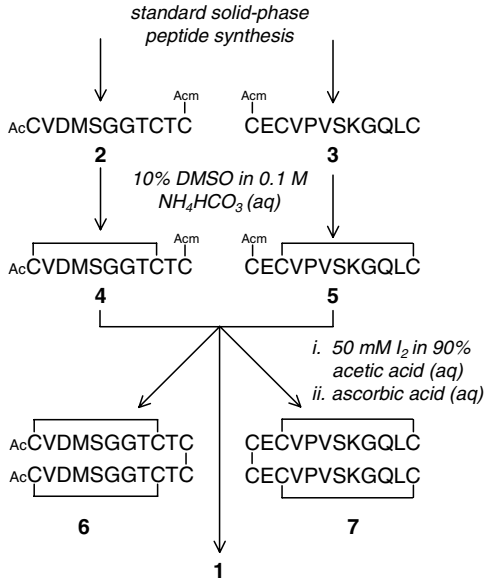
Figure 1. Heterodimeric bicyclic peptide **1**.

In our initial attempts to synthesise **1** (Scheme 1), we employed an Acm/Trt cysteine protection strategy to give the mono-Acm-protected linear peptides **2** and **3**. The free thiols of each of these precursors were oxidised to yield the mono-Acm-protected cyclic peptides **4** and **5**. We anticipated that treatment of an equimolar mixture of **4** and **5** with I_2 would yield the desired heterodimeric bicyclic target compound **1** in addition to the two homodimeric derivatives **6** and **7**. However, using this approach we were unable to obtain more than trace amounts of the desired heterodimeric target peptide **1**. We believe that this is because of the vastly different rates of I_2-mediated Acm cleavage of the monocyclic peptides **4** and **5**. These differing rates meant that the dimerisation reaction yielded initially the homodimer **6**, due to the rapid deprotection of its protected precursor **4**. Upon exhaustion of the monomer **4**, some of the second homodimer **7** then formed sluggishly from its precursor **5**.

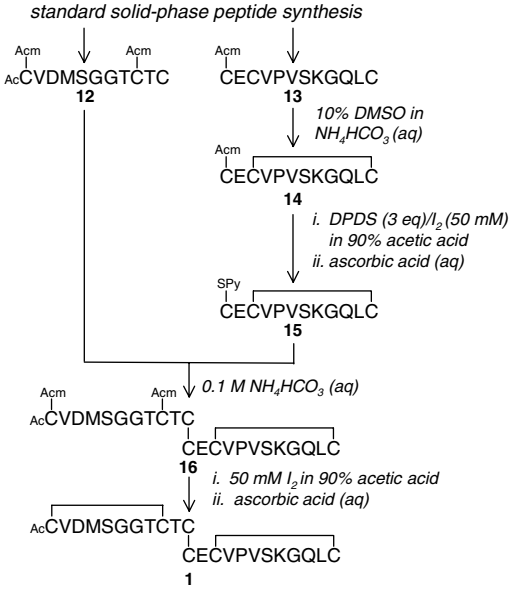
We therefore decided to develop a different strategy with the goal of synthesising solely the desired heterodimer **1**, by examining the synthesis of a model

Keywords: Disulfide; Heterodimer; Bicyclic; Dimerisation; Dipyridyl disulfide; Peptide; BDNF.
* Corresponding author. Tel.: +61 3 8344 8604; fax: +61 3 8344 0241; e-mail: rahughes@unimelb.edu.au

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.08.002

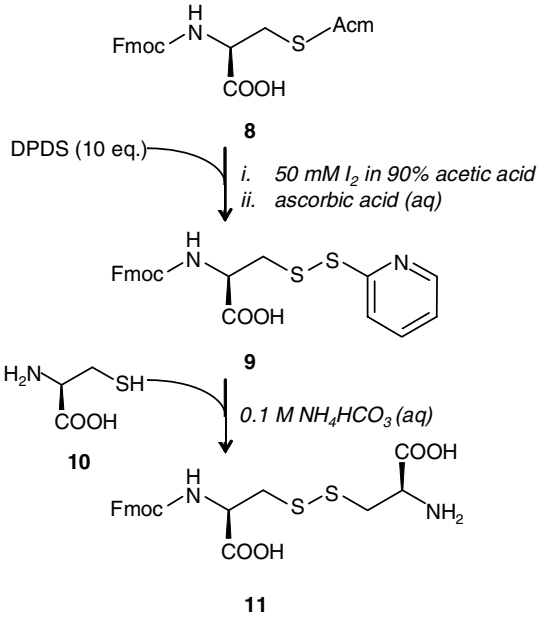


Scheme 1. Synthesis of homo- and heterodimeric disulfide-linked bicyclic peptides.



Scheme 3. Directed synthesis of heterodimeric bicyclic peptide **1**.

compound, the heterodimeric Fmoc-cystine adduct **11** (Scheme 2). After several rounds of reaction optimisation, it was established that treatment of Fmoc-Cys-(Acm) (**8**) with an excess of 2,2′-dipyridyl disulfide (DPDS) in a deoxygenated solution containing 50 mM iodine in acetic acid/water (9:1) yields the SPy derivative of Fmoc-cysteine **9**. This SPy adduct was obtained with approximately 2:1 selectivity over the Fmoc-cystine–Fmoc dimer. The purified activated derivative **9** was then coupled to free cysteine (**10**) to afford the desired heterodimeric adduct **11** (ESI-MS: $[M+H]^+_{calc} = 463.1$; $[M+H]^+_{obs} = 463.0$).[3]



Scheme 2. Synthesis of a model disulfide-linked heterodimer.

We subsequently used this approach to synthesise exclusively the target heterodimer **1** (Scheme 3), without formation of the homodimers **6** and **7**. To achieve this, two unsymmetric monomers were assembled—one bearing two Cys(Acm) and a single free Cys residue **12**, and the other, one Cys(Acm) and two free Cys residues **13**—using standard Fmoc solid-phase techniques.[4] Peptide **13** was oxidised in a solution of DMSO (10%) in aqueous NH_4HCO_3 (0.1 M)[5] to afford the Acm-protected cyclic compound **14**. Using the method established during the synthesis of model compound **11**, Acm-protected peptide **14** was treated with I_2 (50 mM) and a 3-fold molar excess of DPDS in deoxygenated aqueous acetic acid (90%) under a blanket of nitrogen. Under these conditions, the SPy peptide **15** was obtained via an Acm to SPy exchange without the formation of dimer (Fig. 2), in contrast to what we had observed during the synthesis of model compound **9**. The reaction time for this Acm to SPy exchange could be reduced by increasing the molar ratio of DPDS. For example, using 10 equiv of DPDS reduced the time for the formation of **15** to approximately 60 min. However, further increasing the molar ratio of DPDS to 100 equiv gave rise to multiple reaction products. Nevertheless, the shorter displacement time observed using a 10-fold excess of DPDS may be of value in the synthesis of compounds vulnerable to Acm-reattachment, such as Trp-containing peptides.[6]

In the next step of the synthesis of **1**, the Acm-protected heterodimeric monocyclic compound **16** was prepared rapidly and selectively using conditions described previously[7] by mixing the activated cyclic peptide **15** with the free thiol-containing linear peptide **12** in an aqueous solution of NH_4HCO_3 (0.1 M). In the final step, compound **16** was oxidised with I_2 under standard conditions[6] to yield the desired heterodimeric bicyclic target compound **1** (ESI-MS: $[M+H]^+_{calc} = 2376.9$; $[M+H]^+_{obs} = 2377.1$).

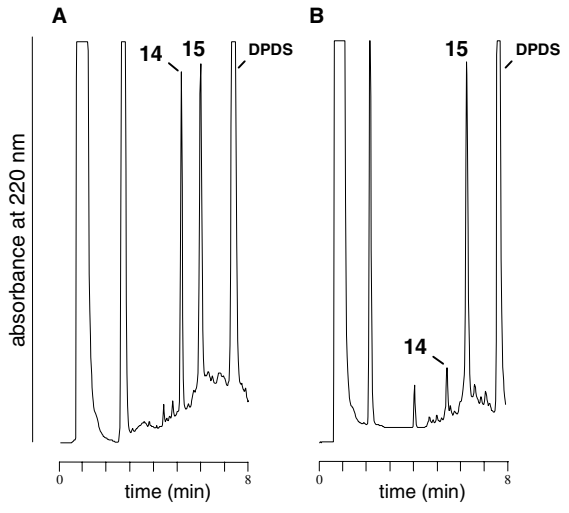


Figure 2. RPHPLC profiles (Alltech™ C_{18} Rocket™ 53 × 7 mm; solvent A: 0.1% TFA in H_2O; solvent B: 0.1% TFA in acetonitrile; 2 mL/min, linear gradient 0–50% B over 8 min) for the formation of **15** from **14** via Acm to SPy exchange. Panel A: 20 min; panel B: 2 h.

In this letter we have described a unique method for the synthesis of a heterodimeric bicyclic peptide. Although methods of DPDS-aided peptide heterodimerisation have previously been reported,[7–10] the one-pot synthesis of an SPy substituted peptide from the corresponding Acm-protected derivative constitutes a novel reaction step. Previous reports describing the synthesis of SPy substituted adducts from other side-chain-protected cysteine residues are available. However, the conditions required for such reactions are relatively harsh—for example, treatment with TFMSA/TFA to remove *t*-butyl[7] or methylbenzyl[10] side chain protection—compared to the milder iodine-mediated conditions described in this letter. Furthermore, it is possible that both methods may be employed together, thereby providing an additional level of orthogonality for the synthesis of multiple cystine-containing peptides.

Acknowledgements

J.M.F. is the recipient of a Melbourne Research Scholarship. We would like to thank A/Prof. John Wade and Dr. Denis Scanlon for helpful discussions during the course of this work.

References and notes

1. O'Leary, P. D.; Hughes, R. A. *J. Neurochem.* **1998**, *70*, 1712–1721.
2. O'Leary, P. D.; Hughes, R. A. *J. Biol. Chem.* **2003**, *278*, 25738–25744.
3. All compounds described in this communication were purified by RPHPLC and characterised by electrospray-ionisation mass spectrometry (ESI-MS).
4. Fields, G. B.; Noble, R. L. *Int. J. Peptide Protein Res.* **1990**, *35*, 161–214.
5. Tam, J. P.; Wu, C.-R.; Liu, W.; Zhang, J.-W. *J. Am. Chem. Soc.* **1991**, *113*, 6657–6662.
6. Kamber, B.; Hartmann, A.; Eisler, K.; Rinikier, B.; Rink, H.; Sieber, P.; Rittel, W. *Helv. Chim. Acta* **1980**, *63*, 899–915.
7. Maruyama, K.; Nagata, K.; Tanaka, M.; Nagasawa, H.; Isogai, A.; Ishizaki, H.; Suzuki, A. *J. Protein Chem.* **1992**, *11*, 1–12.
8. Ruiz-Gayo, M.; Albericio, F.; Pons, M.; Royo, M.; Pedroso, E.; Giralt, E. *Tetrahedron Lett.* **1988**, *29*, 3845–3848.
9. Akaji, K.; Fujino, K.; Tatsumi, T.; Kiso, Y. *J. Am. Chem. Soc.* **1993**, *115*, 11384–11392.
10. Büllesbach, E. E.; Schwabe, C. *J. Peptide Res.* **2001**, *57*, 77–83.