Pergamon

Tetrahedron Letters 39 (1998) 7567–7570

TETRAHEDRON LETTERS

Synthesis of Asymmetric Cystines.

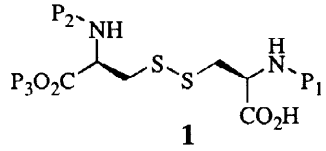
Sheena Zuberi, Angela Glen, Robert C Hider and Sukhvinder S Bansal*

Department of Pharmaceutical Chemistry, King's College London, Manresa Road, Chelsea, London, SW3 6LX.

Received 24 June 1998; accepted 27 July 1998

Abstract: A number of new orthogonally protected asymmetic cystine derivatives have been developed for the synthesis of peptides containing disulphide bridges. Their synthesis was accomplished by $PhI(OAc)_2$ mediated oxidation of cysteine derivatives and using the Npys group to activate the thiol group.

Disulphide bridges are fundamental structural elements in a large variety of biologically active peptides such as hormones, neurotransmitters, growth factors and toxins. These naturally occurring peptides contain single or multiple, intra and/or interchain disulphides. The disulphides impose considerable conformational constraints on these molecules thereby stabilising biologically active conformations. The importance of such cyclic peptides has stimulated increasing efforts towards the development of new thiol protecting groups and suitable strategies for regioselective disulphide bond formation.[1] Currently used methods are based on the formation of the disulphide bridges following solid phase synthesis. This can be achieved by oxidation at high dilution conditions of either the linear peptide to afford the thermodynamically more stable isomer[2] or by selectively protecting cysteine pairs to form the disulphide bridges sequentially.[3] More recently, the group of Barany has demonstrated that disulphide bridges may be formed on the solid support.[4] All of these studies have demonstrated that the formation of the desired disulphide links is dependent on meticulous experimental details is largely sequence dependent and frequently difficult to predict. We propose a novel strategy, whereby an asymmetric cystine derivative, **1** (with two amino groups and one carboxyl group orthogonally protected) could in principle be incorporated into solid phase peptide synthesis to afford cyclic peptides.



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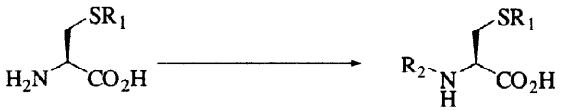
In this approach the carboxyl groups of the asymmetric cystine **1**, can be acylated onto the growing peptide chain and selective release of either P_1 or P_2 would enable further elaboration of the peptide chain. Release of P_3 followed by coupling to the free amino group of the peptide would afford a cyclic peptide. With a second such orthogonally protected asymmetric cystine we could in principle prepare bicyclic peptides. This approach would avoid the use of oxidants (I_2, O_2, $Tl(CF_3CO_2)_3$, $Hg(OAc)_2$) which are generally deleterious to the assembled peptide, particularly for sequences containing tryptophan, tyrosine, methionine and histidine. High dilution conditions necessary for disulphide formation would not be necessary and the desired cyclic peptide would be obtained following cleavage from the peptidyl-resin and purification. More importantly, in this approach intermolecular reactions between the growing peptide chains are not likely be favoured due to the 'pseudo' infinite dilution afforded by the solid support.

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01614-1

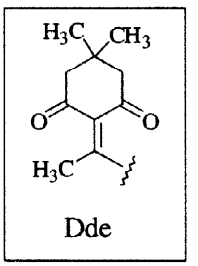
In order to develop this new strategy for the formation of disulphides we required a series of orthogonally protected asymmetric cystine derivatives. In this report we describe the synthesis of protected cysteine derivatives and their conversion into orthogonally protected asymmetric cystines.

The sulphydryl protected amino acids **2** were protected with 2-acetyldimedone and with trimethylsilylethoxycarbonyl chloride (Teoc-Cl) to afford the Nα-1-(4,4-dimethyl-2,6-dioxocyclo hexylidene)ethyl (Dde)[5] derivatives **3a-c** and the Teoc derivatives **3d-e** in good yields, ***Scheme 1***.



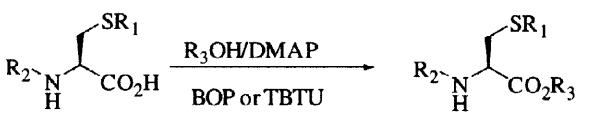
2a $R_1 = CPh_3$
2b $R_1 = CH_2NHCOMe$
2c $R_1 = SCMe_3$
2d $R_1 = CMe_3$

3a $R_1 = CPh_3$ $R_2 = Dde$
3b $R_1 = CH_2NHCOMe$ $R_2 = Dde$
3c $R_1 = SCMe_3$ $R_2 = Dde$
3d $R_1 = CPh_3$ $R_2 = COO(CH_2)_2SiMe_3$
3e $R_1 = CH_2NHCOMe$ $R_2 = COO(CH_2)_2SiMe_3$

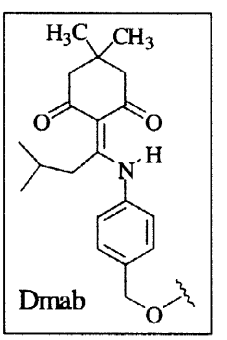


Scheme 1

The carboxyl moiety of the cysteine derivatives **3a-e** and the Fmoc and Boc cysteine protected derivatives of **2** were protected with the trimethylsilylethyl (Tmse) and the recently introduced 4-{N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl] amino}benzyl ester[6] (Dmab) by treatment with the appropriate alcohol, dimethylaminopyridine (DMAP) and coupling agent, Benzotriaol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) to afford the orthogonally fully protected cysteine derivatives **4a-d**, ***Scheme 2***.

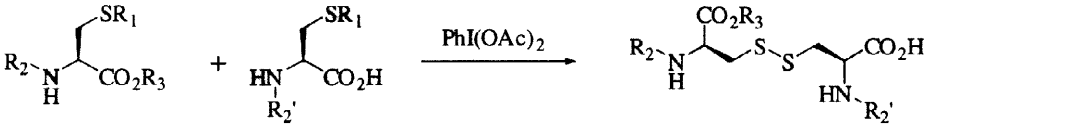


4a $R_1 = CPh_3$, $R_2 = Fmoc$, $R_3 = (CH_2)_2SiMe_3$
4b $R_1 = CPh_3$, $R_2 = Boc$, $R_3 = Dmab$
4c $R_1 = CPh_3$, $R_2 = Dde$, $R_3 = (CH_2)_2SiMe_3$
4d $R_1 = CPh_3$, $R_2 = Boc$, $R_3 = (CH_2)_2SiMe_3$



Scheme 2

Treatment of FmocCys(Acm)-OH with BocCys(Trt)-Tmse **4d** with iodine afforded the asymmetric cystine **5a** along with the symmetrical cystine derivatives. The yield of the desired asymmetric cystine **5a** was low (10%) and purification of the desired product proved difficult. Using diacetoxyphenyl iodide[7] as the oxidant the desired cystine derivative **5a** was prepared in modest yield (25%)



4a-c **3a-c**

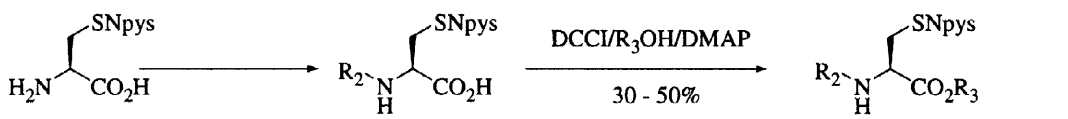
5a R_2 = Boc, R_3 = $(CH_2)_2SiMe_3$, R_2' = Fmoc
5b R_2 = Fmoc, R_3 = $(CH_2)_2SiMe_3$, R_2' = Dde
5c R_2 = Fmoc, R_3 = Dmab, R_2' = Boc

Scheme 3

With this method identified for the preparation of protected asymmetric cystines, we were able to produce three such derivatives in 20 – 40 % yields, ***Scheme 3***. However the procedure was not amenable to scale up and we therefore considered an alternative approach, which would avoid the formation of the symmetrical cystine.

Regioselective disulphide bond formation is possible where one of the sulphydryl protecting groups has a dual role in protecting the thiol and also activating the thiol to nucleophilic attack by a second incoming free thiol. In this approach, S-methoxycarbonylsulphenyl (Scm)[8] derivative has been utilised where the driving force for the reaction is the loss of inert carbonylsulphide. Another method of activating the thiol involves reaction with aromatic sulphenyl halides such as 2-nitrophenylsulphenyl[9] (Nps) and 3-nitro-2-pyridinesulphenyl[10](Npys).

We chose to utilise the Npys group since the disulphide bond formation can take place over a wide pH range in aqueous buffer and it can be monitored by the spectroscopic release of 3-nitro-2-pyridinethiol. 3-Nitro-2-pyridinesulphenyl chloride, (Npys-Cl) was prepared in good yield according to literature procedure.[10] HCys(Npys)OH, **6** was obtained by treatment of cysteine with Npys-Cl. The corresponding Nα-protected derivatives **7a-c** were successfully prepared in good yields. Finally, the corresponding esters were prepared with Dmab and Tmse **8a-c** albeit in modest yields. ***Scheme 4***.



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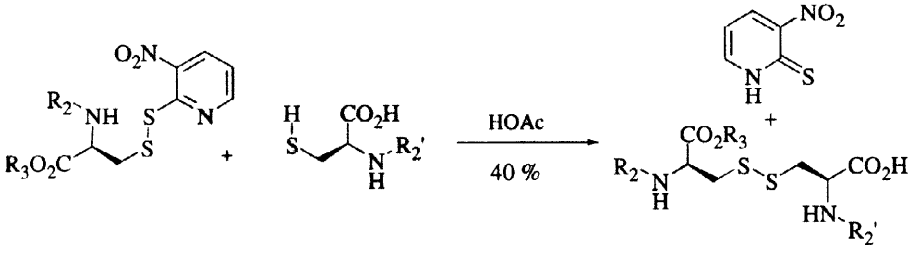
7a R_2 = Boc
7b R_2 = Fmoc
7c R_3 = Dde

8a R_2 = Boc, R_3 = $(CH_2)_2SiMe_3$
8b R_2 = Fmoc, R_3 = $(CH_2)_2SiMe_3$
8c R_2 = Dde, R_3 = $(CH_2)_2SiMe_3$

Scheme 4

For the regioselective disulphide bond formation the free thiol **9a-c** was obtained by treatment of DdeCys(Trt)OH and FmocCys(Trt)OH with TFA or by reduction of the S-tbutylsulphenyl cysteine derivative with tributylphosphine. The free thiol was not purified to avoid oxidation and it was used immediately.



8a-c **9a** R_2 = Fmoc; **9b** R_2 = Dde; **9c** R_2 = Boc **5a-c**

Scheme 5

The free thiol component **9a-c** was treated with the Npys protected cysteine derivatives, **8a-c** to afford the asymmetric cystines **5a-c, Scheme 5**. At high pH, this reaction resulted in a number of unidentified by-products whereas at low pH the reaction was rather slow. Optimal conditions for the formation of the asymmetric disulphide were found at pH 6 and using these conditions three asymmetric disulphide were prepared in good yield (40%) and fully characterised by HPLC, FAB MS and NMR. In conclusion we have developed a facile synthesis of a number asymmetric cystine derivatives which are currently being evaluated for the utility in solid phase peptide synthesis.[11]

Acknowledgements: *We would like to thank the EPSRC for financial support(GR/K02435) and Dr Peter White (Calbiochem-Novabiochem UK Ltd) for a generous gift of Dmab.*

Abbreviations: *Amino acids and peptides follow the IUPAC-IUB nomenclature (Eur. J. Biochem. 1984, 9-37); Teoc, trimethylsilylethoxycarbonyl; Tmse, trimethylsilylethyl; Fmoc, 9-fluorenylmethoxycarbonyl; Boc, t-butoxycarbonyl; TFA, trifluoroacetic acid; Trt, trityl.*

*e-mail: sukhi.bansal@kcl.ac.uk

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11 Recently it has been reported that some premature cleavage of the Dde group takes place during Fmoc deprotection, N to N' migration of the Dde group has also been reported and the authors advocate the 1-(4,4-dimethyl-2-dioxocyclohexylidene)-3-methylbutyl(Ddiv) group (Chhabra, S.R.; Hothi, B.; Evans, D.J.; White, P.D.; Bycroft, B.Y.; Chan, W.C. *Tetrahedron Lett.* **1998**, 39,1603). We are currently investigating this group in the synthesis of asymmetric cystines.