Tetrahedron Letters,Vol.28,No.40,pp 4637-4640,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

PEPTIDE SYNTHESIS BY PRIOR THIOL CAPTURE—V. THE SCOPE AND CONTROL OF DISULFIDE INTERCHANGE DURING THE ACYL TRANSFER STEP

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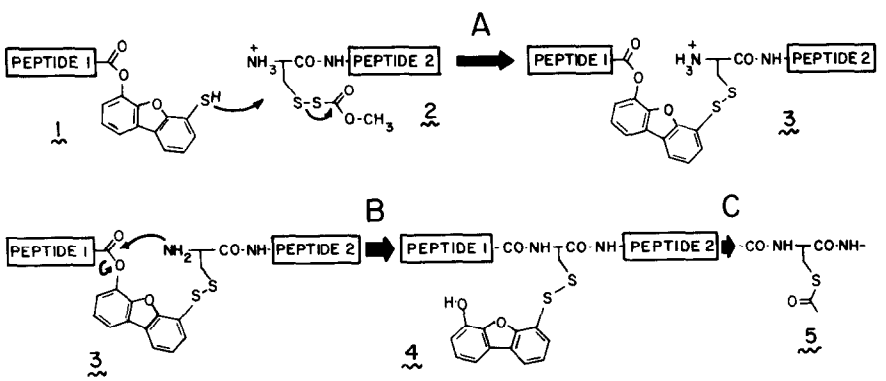
Abstract: Disulfide interchange in DMSO, during amide formation by prior thiol capture is reduced to less than 3% at low concentrations (<10 M) of substrate in the absence of air and light, and in the presence of 2.5 to 10 mole % $AgNO_3$. The rate and selectivity of the exchange process were assessed by reacting 6 with a thiol; 7 was formed almost exclusively with a rate constant on the order of 10 to 10 M s .

As seen in Scheme I, the thiol capture strategy involves linkage of a pair of peptide fragments 1 and 2 with the unsymmetrical disulfide bond of 3, using the Scm group of Brois, Hiskey and Kamber.[1] Amide-forming intramolecular O,N-acyl transfer then occurs in the aminolysis-accelerating solvent DMSO. Since the key to the overall strategy is the use of a relatively unactivated phenyl ester 3 for the acyl transfer step, it was essential to define the degree to which disulfide interchange provides a competing series of side reactions during

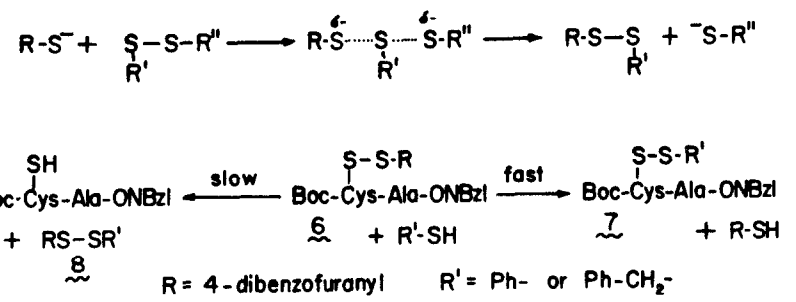
SCHEME I



the 12 hr period required to bring a typical conversion 3 → 4 to 90% completion.[2] At the beginning of the acyl transfer process, the disulfide 3 has the potential for equilibrating with two symmetrical disulfides. Mid-way through the acyl transfer, a worst-case analysis involves the equilibration of a mixture of 3 and 4 with four symmetrical and four unsymmetrical disulfides. In this communication, we report rate and product studies for disulfide interchange reactions in the dipolar aprotic solvent DMSO, pertinent to the optimization of the general conversion 3 → 4.

Effects of substituents on the position of equilibria for disulfide interchanges are small in protic media[3,4] and should remain so in DMSO. Rate factors therefore provided the hope for controlling the reaction of unwanted disulfides. Under mild conditions in protic media, most disulfide exchange reactions proceed by an S_N2-like thiolate displacement mechanism of Scheme 2. For aqueous reactions the available rate data have been recently correlated with pKa values for the thiols that correspond to the three sulfur atoms of the Scheme.[4] Given a pKa difference of 8.7-6.5 = 2.2 for the cysteine and arenethiol functions of the disulfide 3, one expects a rate ratio in water of only 2-10 for the two substitution reactions of a thiolate anion with 3. As tests of this point we examined the disulfide 6; first, alone in DMSO solution, and second, in the presence of an added thiol.

SCHEME 2



To determine the rapidity of solvent-induced interconversion with the corresponding pair of symmetrical disulfides, 6 was allowed to remain at 25°C in DMSO for 19 h; 98 % was unchanged and 2 % was converted to symmetrical disulfides under these conditions. (No exchange was seen in MeOH or MeCN.) Thus, while DMSO may induce a slow exchange, this process should not complicate a typical conversion of 3 → 4. Preparatory to a study of the reactions of 6 with Ph-SH and $PhCH_2$-SH we synthesized the series of five disulfides that could be formed from 6 and each thiol and found HPLC conditions that could resolve and assay a mixture of 6 with each series of disulfides. Reactions of 6 were carried out with a 10 to 100-fold excess of either thiol in DMSO at 25°C. Within seconds at [thiol] = 10^{-2} to 10^{-3} M, a single rapid disulfide interchange occurs by displacement at the cysteine sulfur, with formation of 4-dibenzofuranthiol and 7. The observed ratio of the two unsymmetrical disulfides 7 and 8 that can be generated directly from 6

is 99:1. Even allowing for uncertainties[4] in the estimating procedure, we conclude that the shift from water to DMSO results in a remarkable and gratifying increase in the selectivity of the exchange reaction.

Although the mechanism of Scheme 2 almost certainly applies to these reactions, it seemed important to establish the rate law and to provide evidence for the intermediacy of thiolate anions in the exchange process.[5] Reaction of 6 (8.7×10^{-4} M) with $PhCH_2SH$ (1.9×10^{-2} and 1.0×10^{-1} M) established the rate equation to be $k[6][PhCH_2SH]^{1/2}$, with $k = 8.2\times10^{-2}M^{-1/2}s^{-1}$. A similar study with PhSH (2.5, 4.9, 9.8 mM) gave an identical rate law with $k = 8.8\times10^{1}M^{-1/2}s^{-1}$. The dependence on the square root of thiol concentration is expected if the thiol undergoes dissociation in DMSO to form a thiolate anion. From the work of Ritchie[6], the dissociation of weak acids is known to follow a simple law of equilibrium in DMSO solutions, and the pK_a of benzenethiol shifts from an aqueous value of 6.6 to 10.3 in DMSO. Using this value, one can estimate the rate constant k_2 for the reaction of the thiolate anion with 6 to be $1.2\times10^{7}M^{-1}s^{-1}$. An independent estimation of this rate constant was obtained by measuring the rate of reaction of 6 with Ph-SH in DMSO containing a 1,1,1-trifluoroethylamine buffer. The rate law under these conditions was found to be $k[6][Ph\text{-}SH][CF_3CH_2NH_2]/[CF_3CH_2NH_3^+]$; the pk_a value[7] of 5.8 for $CF_3CH_2NH_3^+$ then gives a strongly salt-dependent value for k_2 of $5\times10^{6}M^{-1}s^{-1}$ at at ammonium ion concentration of 2.10^{-2} M. In view of the uncertainties in extrapolating to minute thiolate concentrations, the agreement within a factor of two of these rate constants is satisfactory and in support of the general mechanism. Not surprisingly the stability of the nucleophile $R\text{-}S^-$ and the leaving group $R''\text{-}S^-$ are the major determinants of the rates of disulfide interchange in DMSO as in water.[4] However, in the absence of protic stabilization, the nucleophiles are more difficult to generate (ΔpK_a of 4-6), more subject to stabilization by internal electronic effects, and more reactive. The reaction also appears to be more sensitive to the stability of the leaving group, and this is the likely explanation for the high selectivity for reaction at the cysteinyl sulfur.

From the above results it is clear that control of excess thiol is the key to avoiding disulfide interchange, and that trace amounts of thiol such as those that might persist from the reaction 1 + 2 → 3 are efficient catalysts, particularly in the presence of tertiary amine bases that are the normal components of the reaction 3 → 4. It was important to demonstrate that the above results are consistent with typical patterns of disulfide interchange that are observed during practical conversions 3 → 4, and that practical procedures exist for scavenging traces of thiols. Studies of a variety of preparative reactions 3 → 4 have shown that the important variables for controlling disulfide interchange are low substrate concentration ($<10^{-3}$ M) and the absence of air and light. Typically, 3 (Peptide 1 = Z-Pro-Phe-Thr(OtBu)-, Peptide 2 = -Gly-Gly-Ala-ONBzl), 5×10^{-4} M in DMSO, 25°C, 20 h, N_2, dark, gave an 8.5:1 mixture of two components which were identified as 4 and the O-ester of 4 and Z-Pro-Phe-Thr(OtBu)-OH. The latter product is almost certainly formed by S-S interchange, catalyzed by a trace of 1. Addition of $AgNO_3$ (2.5 to 10 mole%) to the reaction mixture before acyl transfer suppressed the

formation of the latter byproduct to less than 3%. Results to be reported in detail elsewhere show that the addition of silver ion[8] in this concentration range (up to 5 mM) results in no detectable side reactions or complications.[9]

ACKNOWLEDGEMENTS Financial support from the N.I.H., Grant GM 13453 is gratefully acknowledged. We thank Dr. C. Costello of Prof. K. Biemann's laboratory for FAB mass spectra.

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9. Although phenylmercuric acetate is also an efficient thiol scavenger, Hg-induced SS cleavage has occasionally been observed. Thus for the above-cited Thr-Cys coupling, product 4 + PhHgOAc yielded 4-phenylmercurithio-6-dibenzofuranol (FAB MS, NMR).

(Received in USA 7 May 1987)