Tetrahedron Letters Vol. 22, pp 181 - 184 0040-4039/81/0115-0181$02.00/0

MODELS THAT DEMONSTRATE PEPTIDE BOND FORMATION BY PRIOR THIOL CAPTURE I. CAPTURE BY DISULFIDE FORMATION

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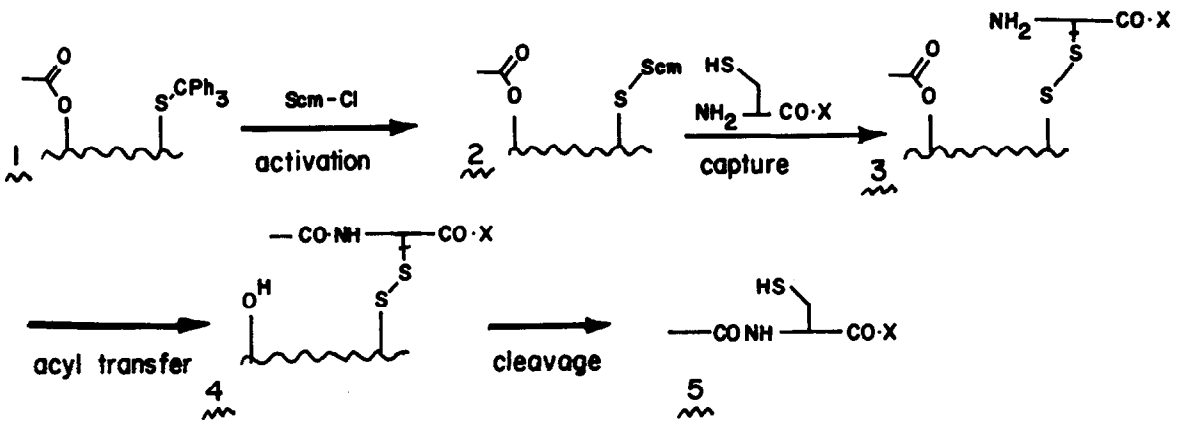
Abstract: Unsymmetrical disulfides formed from L-cysteine esters and o-Cbz-L-alaninyloxybenzenethiol 9, 2-Cbz-L-alaninyloxy-5-chlorophenylmethylenethiol 8, 4-acetoxyxanthenylmethylene thiol 10, and 1,5-diacetoxy-2-methyl-3-methoxy-4-thioxanthone 11 are observed to undergo intramolecular O,N-acyl transfer in yields up to 60%, with accompanying disulfide interchange. The significance of these results for a general amide forming strategy of prior cysteine capture are discussed.

Peptide synthesis currently lacks reagents that can be used to form amide bonds reliably between peptide fragments of high molecular weight. We have previously outlined a new strategy of prior amine capture[1] in which amine and active acyl components are linked in a capture step which precedes amide bond formation, which then proceeds intramolecularly. Provided the capture step occurs cleanly and rapidly at high dilution and in solvents that inhibit unproductive association of peptide fragments, this strategy offers the potential for replacing the enthalpic activation of a highly electrophilic acyl carbonyl (which becomes an inefficient and side-reaction-prone acylating agent at high dilution) with a proximity-based entropic activation for acyl transfer.

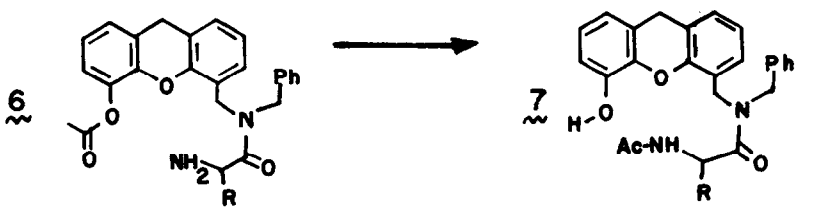
Of the functional groups that appear in peptides, the thiol function of cysteine offers the greatest potential for meeting the above capture requirements. We plan to use two reactions of thiols, with mercury derivatives and with the activated, unsymmetrical disulfide-forming S-Scm[2], to form covalent bonds to the sulfur of cysteine. In this communication we demonstrate the feasibility of the reaction sequence shown in Scheme 1, which if realizable in practical

form, would be applied in the final stages of the synthesis of a large peptide or protein to form bonds to amine components bearing N-terminal cysteine residues.

Scheme 1 Prior Thiol Capture by Unsymmetrical Disulfide Formation

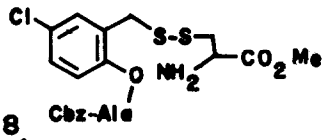


The most problematic step of this scheme is the acyl transfer 3 → 4, which must proceed through the intermediacy of a medium-sized ring. Elsewhere[3] we have reported rapid and efficient acyl transfers for structures such as 6 → 7 and have discussed the criteria for transition state structure that have directed our choice of geometry for this and other starting materials; these include a trans, anti orientation of $^{\alpha}$C-CO and N-$^{\alpha}$C bonds about the forming C-N bond, a rigid spacing unit, minimal van der Waals interactions and an absence of vacant spaces in the proposed transition state of acyl transfer.



A number of structures which meet all of these conditions except rigidity have failed to exhibit detectable O,N-acyl transfer to captured cysteine residues.[4] With the assumption of a relatively rigid geometry for the S-S function and a dihedral angle close to 90°,[5] we find that structures 8, 9, 10, and 11 appear to meet the above criteria.

In DMSO solution over 20-40 h, benzyl derivatives 8 and 10[6] formed product mixtures in which the amides resulting from O,N-acyl transfer could be demonstrated by ^{1}H NMR, HPLC, and TLC. Products of disulfide interchange were detected as well, and the amide products were found to be unstable under the reaction conditions and gave similar mixtures over the course of days.



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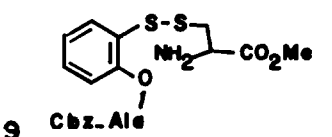

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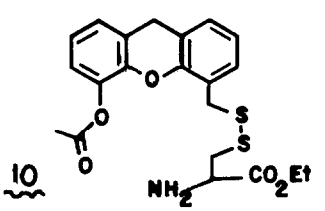

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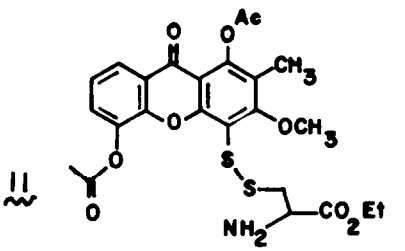

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More definitive results were obtained with 9 and 11[6], which were found to undergo O,N-acyl transfer with concentration-independent rates in DMF and DMSO. Half times for 11 at 10^{-4} M concentration and 25° were 2.7 h in DMSO and ca 28 h in DMF. A preparative run on 5 mg scale (0.05 M in DMSO solution) for 7.5 h gave 43% of N-acetylcysteine product, which was characterized spectroscopically. In order to provide a measure of the efficiency of acyl transfer, the concentration of ethyl S-benzyl cysteine required to compete equally with the internal nucleophile was estimated from the reactivity of 1,3-dimethoxy-2-methyl-5-acetoxyxanthone to be 0.6 M. This can be regarded as the effective local concentration of the intramolecular amine nitrogen at the 5-acyl carbon of 11.

When 9, radiolabeled with ^{14}C at the alanine 1-C, was allowed to react in DMSO solution at concentrations of $1\text{-}3\times10^{-4}$ M for 80-90 h, solvent removal, DTT (dithiothreitol) cleavage, and isotopic dilution with product demonstrated 50-60% yields of Cbz-L-Ala-L-Cys-OCH_3. Analysis of this mixture and that from reactions in DMF demonstrated the products of disulfide interchange; in hydroxylic solvents 9 was found to undergo solvolysis of the acyl-oxygen bond with little or no detectable amide formation.

These model studies have demonstrated assembly of separate molecules of an acylating agent and a cysteine derivative to achieve effective local concentrations of amine at the acyl carbon that approach 1 M. Although they provide working examples for the most problematic features of Scheme 1, they point up two difficulties that must be resolved before a practical method for coupling peptides can be based on this scheme. First, the intramolecular amide-forming reactions of 8, 9, 10 and 11 occur so slowly that disulfide interchange reactions are competitive. Second, the reactions of cysteine derivatives with the Scm-bearing precursors of 8, 9, 10 and 11 do not occur cleanly and do not approach quantitative yields, unlike the corresponding reactions of cysteine species with Scm-bearing cysteine derivatives. Studies to resolve these problems are in progress and will be reported subsequently.

Acknowledgment: Financial support through

References:

1. D.S. Kemp, J.A. Grattan, and J. Reczek, J. Org. Chem., 40, 3465 (1975).
2. S.J. Brois, J.F. Pilot, and H.W. Barnum, J. Amer. Chem. Soc., 92, 7629 (1970).
3. D.S. Kemp, Y.A. Hsieh, D. Kerkman, S-L. Leung, and G. Hanson, "Peptides-1978", I.Z. Siemion and G. Kupryszewski, eds., Wroclaw University Press, Wroclaw, Poland, 1979, P 147.
4. D. Kemp and G. Hanson, unpublished observations.
5. A. Hordvik, Acta Chem. Scand., 20, 1885 (1966).
6. Species 8, 9, and 10 were prepared by reaction of Boc-L-Cys-OH esters with Scm derivatives, followed by treatment with HCl in dioxane and liberation with $NaHCO_3$; species 11 was formed by deblocking of the product formed by treatment of the xanthone thiol and a 10-fold excess of Boc-Cys-OEt with I_2. This disulfide could not be formed from the corresponding Scm derivative.

(Received in USA 25 September 1980)