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PEPTIDE BOND FORMATION USING AN ENZYME MIMICKING APPROACH

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Abstract: *A man-made enzyme-model based on a concerted proton transfer (bifunctional catalysis), and mimicking the non-ribosomal peptide synthesis occurring in some microorganisms, was developed.*

During the 1970s the biosynthetic pathway occurring in some microorganisms and leading to peptides such as *Tyrocidine* and *Gramicidin-S* was completely clarified.[1] *Gramicidin-S* biosynthesis, for example, does not rely on the presence of ribosomes or tRNA. Two enzymes, a "light enzyme" (MW ca.100,000) and a "heavy enzyme" (MW ca.280,000) are involved, and the biosynthetic sequence appears to be much simpler and more primitive than the usual ribosomal protein biosynthesis.[2,3a] The fundamental steps of the biosynthetic sequence are the following: (a) thiolesters are synthesized using ATP-activated α-aminoacids and thiol groups of the enzyme (b) thiolesters are cleaved by enzyme-catalyzed intramolecular aminolysis with consequent formation of a peptide bond and of a free thiol group (c) this process is iterated with consequent formation of the polypeptide chain. This pathway is used by Nature for the production of relatively small peptides, consisting of about 10 residues.

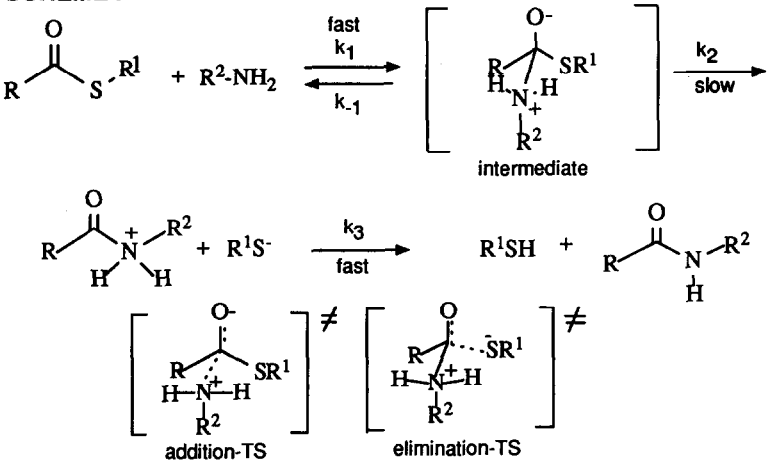
Here we report on a man-made enzyme-model mimicking this sequence of reactions.

Previous attempts to mimic the thiolester mediated non-ribosomal peptide synthesis[4,5] substantially differ from the work reported here. Particularly, in the Koga's approach[5] the catalyzed step is the thiolester synthesis, through crown ether complexation of the aminoacid-$NH_3^+Br^-$ group and thiolysis of the aminoacid p-nitrobenzoate. In our work thiolesters are easily synthesized by regular chemistry involving carboxylic acid activation through mixed anhydrides (e.g. DCC,4-PP; DPPA; DEPC; etc.)[6] in a way which is quite similar to the biosynthetic ATP activation. Our efforts are directed to catalyze the intramolecular thiolester aminolysis step, which is the key reaction for peptide bond formation.

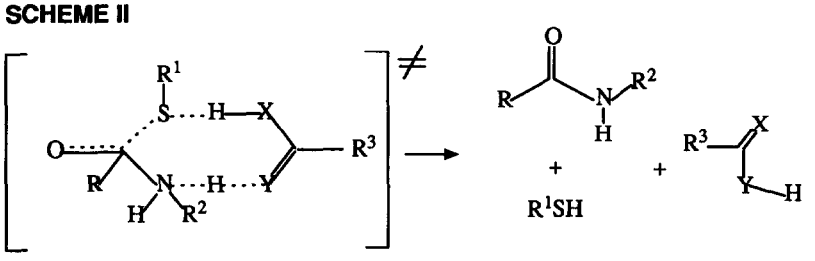
Ester and thiolester aminolysis has been studied in some detail, including mechanism and kinetics.[7] On the basis of the results reported in the literature[7b,c,d,e] the reaction mechanism can be tentatively described as shown in Scheme I. In most cases, when R^1 is not particularly electron-withdrawing, the rate determining step is the thiol elimination. Therefore the reaction appears to be controlled by the elimination-TS (see Scheme I). Although thiolesters are high energy substrates and thiolester aminolysis is definitely a thermodynamically favored process, the reaction is very sluggish (a rate constant of 1.5×10^{-5} $sec^{-1}M^{-1}$ was reported for a simple intermolecular case)[5d] because of the high activation energy of the thiol elimination process. One way to decrease this activation energy is to change R^1 from an alkyl group to a p-nitrophenyl group. Another way is to catalyze the process by proton-transfer at the TS level from the ammonium cation to the sulfide anion. This concerted proton-transfer assistance (bifunctional catalysis) has been used to catalyze all the alkyl thiolester inter- and intramolecular aminolyses known in the literature[4,5,8] (Scheme II).

The most popular bifunctional catalysts are bicarbonate, the monoanion of phosphate, substituted phosphonates, methyl arsonate in aqueous solutions, and 2-hydroxypyridines (e.g. 2-pyridone) or carboxylic acids (e.g. pivalic acid) in aprotic solvents.[3b,7h,9] A typical thiolester aminolysis is usually conducted in benzene at room temperature in the presence of 0.15 M pivalic acid and 0.15 M Et_3N. Usual rate constants are around $2\text{-}6x10^{-5}$ sec^{-1} for the intramolecular cases.[5]

SCHEME I



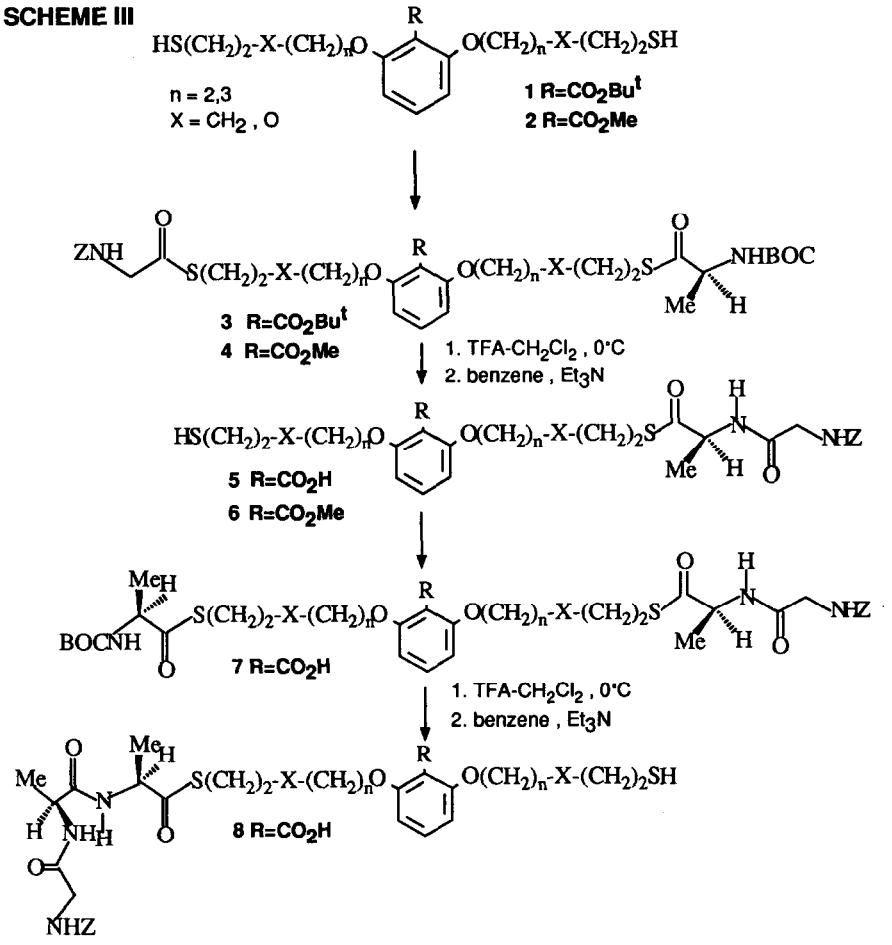
SCHEME II



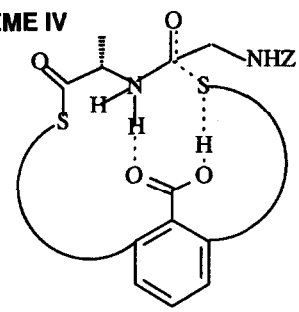
We have developed a bifunctional catalyst covalently bound to the same molecule bearing the thiol groups and incorporating the following features: (a) a bifunctional acid-base catalyst for the thiolester aminolysis rate acceleration (b) two thiol-containing arms mimicking the "swinging arms" of the enzyme[1,2,3] (c) symmetry elements so that the process can be iterated with consequent formation of the polypeptide chain.

Starting from commercially available 2,6-dihydroxybenzoic acid, dithiols **1** and **2** (Scheme III) were easily synthesized[10] and monoacylated with Z-Gly *via* DCC/4-PP[6] or DEPC[6] activation (50-55%). A second aminoacid (BOC-Ala) was then introduced (DPPA/DMF,[6] 90-95%) to give compounds **3** and **4**. BOC and the t-butyl ester of **3** were both removed (1:1 TFA-CH_2Cl_2, 0°C, 100%), the solvent evaporated and Et_3N (1.5 mol.equiv.) was added (to free the primary amine from CF_3CO_2H) in benzene under high dilution conditions ($1x10^{-3}$ M).

SCHEME III



SCHEME IV



Peptide bond formation occurs, as depicted in Scheme IV, catalyzed by rapid proton transfer through an eight-membered ring *via* the properly positioned carboxylic acid. Experiments with n=3, R=CO_2H gave dipeptide **5** (80% isolated yield) with rate constants = $8x10^{-5}sec^{-1}$ (X=O, $t_{1/2}$= 2 h and 25 min) and $5x10^{-5}$ sec^{-1} (X=CH_2, $t_{1/2}$= 3 h and 50 min), and with ***at least a 10^3-fold rate acceleration*** in comparison with the correspondent non catalyzed process yielding **6** from **4** [n=3, X=O or CH_2, R=CO_2Me, ***no reaction product***

detected after 6 months ($k < 5x10^{-8}$ sec^{-1})]. This acceleration is still relatively small, compared to those reported in other cases (e.g.$1.5x10^5$),[11] but shows the feasibility of the process. The reaction is also 4-8 fold faster than the analogous process **4** → **6** (n=3, R=CO_2Me) run in the presence of 0.1 M pivalic acid and 0.1 M Et_3N, characterized by rate constants = $2x10^{-5}$ sec^{-1}(X=O) and $6.2x10^{-6}$ sec^{-1} (X=CH_2).

Important structure and reaction parameters are the following: (a) bifunctional catalysis is inhibited by more polar solvents (e.g. CH_2Cl_2, CH_3CN, DMF, DMSO) (b) weaker (Pyridine, 2,6-Lutidine) or stronger (DBU) amines are less effective than Et_3N (c) more than 2 or less than 1 mol. equiv. of Et_3N tend to slow down the reaction, because of carboxylate formation (more than 2) or incomplete CF_3CO_2H exchange (less than 1) (d) the presence of oxygen in the arms has some relevance: there is a 1.6-3.2 fold rate acceleration on going from n=3, X=CH_2 to n=3, X=O (e) CPK models suggest that the 21- and 23-membered macrocyclic transition states, corresponding to n=2,3 (Scheme III, IV), give the best fit for the bifunctional catalysis to occur with the proper orientation (f) no significant rate difference was observed between the n=2 and n=3 series (e.g. n=2, X=O, R=CO_2H, k=$7x10^{-5}$ sec^{-1}).

A new BOC-aminoacid (e.g. BOC-Ala) can then be added (DPPA/DMF,85%) to give **7** (n=3, X=O). Unfortunately the second intramolecular aminolysis (Scheme III) is complicated by the unfavorable steric hindrance at the α-carbon of the acyl group (CHMe vs. CH_2 of the first aminolysis)[12] with consequent formation of the desired tripeptide **8** (n=3, X=O) in low yield (35%), together with dipeptide **5** and Ala-Ala-S-(Spacer)-S-Ala-Gly-Z both deriving from intermolecular aminolysis. We are currently working on a new macrocyclic model characterized by a reduced conformational freedom of the two arms, which should overcome this problem.

The past two decades have seen an enormous interest in developing chemical models of enzymatic catalysis. The high efficiency of enzymatic catalysis has been generally attributed to typical chemical mechanisms, operating under the especially favorable conditions present in the enzyme-substrate complex. Several mechanisms - general acid, general base, nucleophilic, and bifunctional catalysis - appear to be particularly relevant to enzymes. Models of each have been studied with the goal of approximating the catalytic efficiency observed in enzymes.[13] We have reported here on a chemical model for peptide synthesis based on a concerted proton transfer (bifunctional catalysis).

Notes and References

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