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Development of Synthetic Catalysts for Peptide Bond Cleavage
Synthesis and Complete Kinetic Analysis of Compounds 6A, 7A, 8A

12 PERSONAL AUTHOR(S)

Mertes, Kristin Bowman, P.I., Mertes, Mathias Peter, Co-P.I.

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Synthetic mimics for carboxypeptidase A will by synthesized and the structural and chemical factors responsible for catalytic peptidase activity will be probed. Ditopic macrocyclic receptors have been designed which incorporate the salient features of the enzyme analog, namely high affinity complex formation, general base and general acid catalysis, and covalent catalysis. Once synthesized the resulting macrocycle-metal ion complexes should non-specifically promote the hydrolysis of C-terminal peptide bonds. The initial macrocycles will have several types of coordination sites: nitrogen-containing heterocycles, ammonium and ether oxygens. One side of the ditopic receptor will preferentially bind zinc(II) ion, the other the peptide substrate.

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Date: August 6, 1988

ANNUAL PROGRESS REPORT ON CONTRACT N00014-86-K-0862 TASK NO. 44g016

PRINCIPAL INVESTIGATOR: Kristin Bowman Mertes

CO-PRINCIPAL INVESTIGATOR: Mathias P. Mertes

CONTRACT TITLE: Development of Synthetic Catalysts for Peptide Bond Cleavage: Synthesis and Complete Kinetic Analysis of Compounds 6A, 7A, 8A

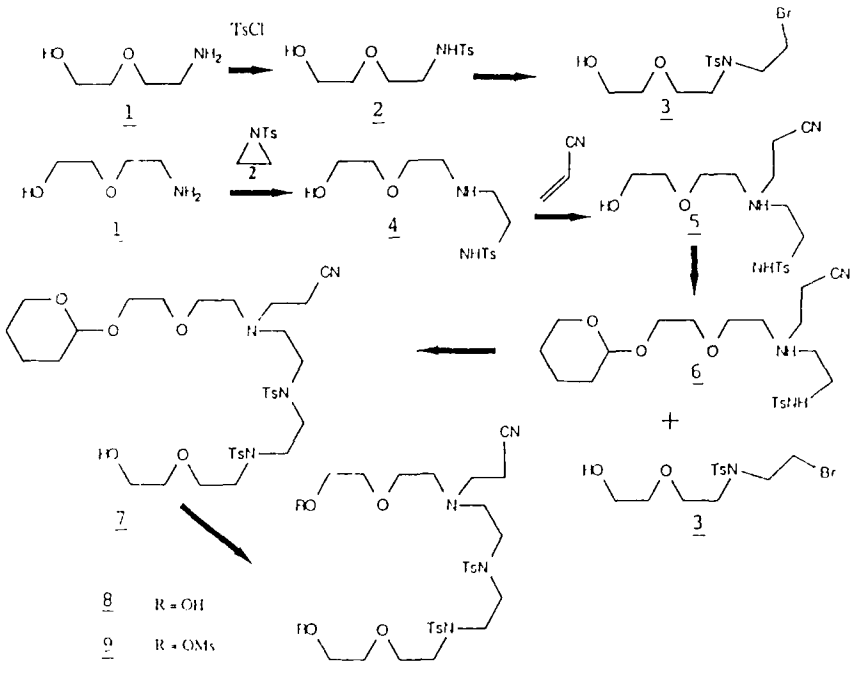
START DATE: 6 August 1986

RESEARCH OBJECTIVE: To synthesize three macrocyclic ditopic receptors as mimics for carboxypeptidase A, i.e. as hydrolytic catalysts for ester and amide bonds alpha to a carboxylate group.

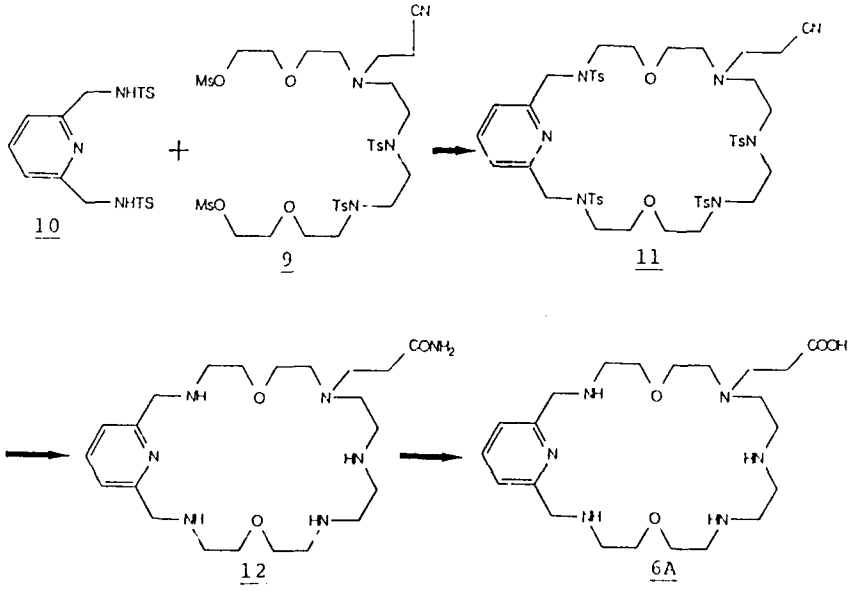
PROGRESS (YEAR 2): The report for the first year concentrated on the most optimal synthetic route for the preparation of the azoxychain representing the 'eastern' portion of the three target compounds shown in Figure 1. One of the major problems encountered was a polymerization reaction where we could not adequately control the number of units of the tosylaziridine that added to the azoxychain (8, Scheme 1, 87 report). This was complicated by the finding that the three major isomers were not easily separated. A second problem was the cyclization of the activated derivative (14, Scheme 1, 87 report) to give the morpholine.

These problems have now been solved using a convergent sequence as shown in Scheme 1. In this method the two halves, 3 and 6 are combined to give 7. This avoids the difficulties in extension of 5 which could not be controlled. While the overall yield is not good, simple starting materials combined with easier purification of the intermediates have afforded a reasonable amount of 8.

Reaction of 10 (87 report) with 9 afforded a reasonable yield of the protected macrocycle 11. Deprotection of 11 was a critical problem for several months until precise reagent quantities and temperature control were found. The problem encountered was that the side chain was being eliminated as acrylonitrile (Retro-Michael reaction). The amide 12 was characterized by ^{1}H and ^{13}C NMR, mass spec, and elemental analysis. Acid catalyzed hydrolysis of the amide to the acid 6A gave a product that had the correct NMR. However, we are concerned about the fact that we cannot get the molecular ion in the mass spec; we only see the $M^{+} + 1 - H_2O$. While the fragmentation pattern supports the structure the mass spec peak could also arise from a cyclic amide formed in the conversion of 12. IR data shows a carbonyl shift from 1670 (amide) to 1720 (COOH?). Further work will be done to assure that the acid 6A is the product and not a lactam by cyclization.

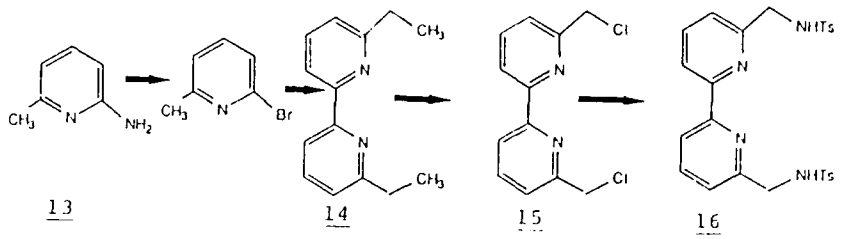


Scheme 1. Convergent route for the preparation of 9, the 'eastern' half of 6A, 7A, 8A.



Scheme 2. Synthesis of 6A.

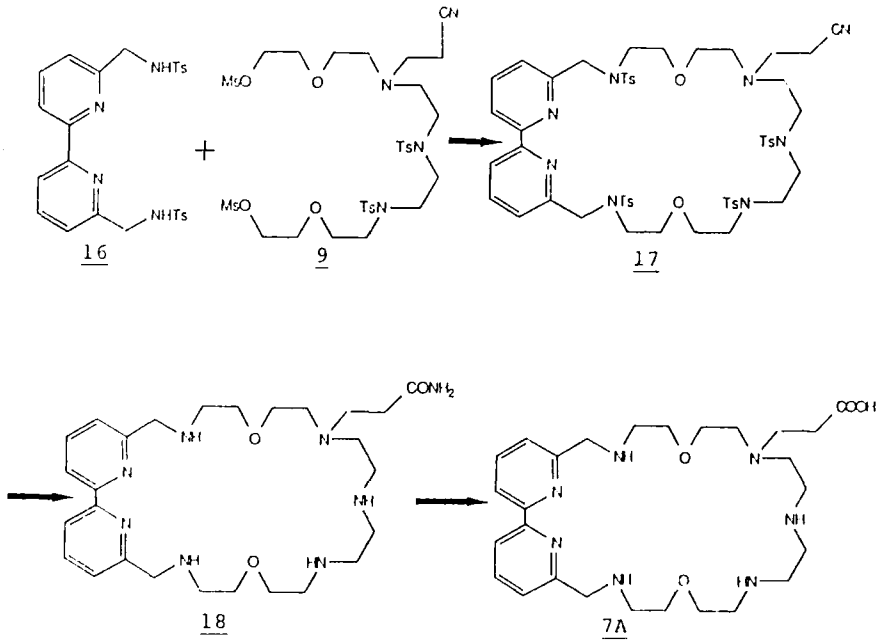
The 'western' half of compound 7A was prepared in low overall yield as shown in Scheme 3 starting with 13.



13 14 15 16

Scheme 3. Synthesis of the bipyridyl derivative 16, the 'western' portion of 7A.

Using the methods worked out for the first target compound, 7A was prepared by reaction of 16 with the activated derivative 9 (Scheme 4). Again, controlled temperature and reaction monitoring were essential to minimize polymer formation. Conversion of 17 to the amide 18 proceeded as before. However, the amide hydrolysis to 7A again afforded a product without the mass spectral molecular ion, only the dehydration product. Steps will be taken to verify that the structure is 7A, not a lactam.



16 9 17 18 7A

Scheme 4. Synthesis of 7A

WORK PLAN (YEAR 3)

1. Structures 6A and 7A will be established.

2. Compound 8A will be synthesized using the methods worked out during the first two years.

3. Metal (Zn^{2+}) complexation with these macrocycles will be studied.

4. The assay for peptidase and esterase activity using the macrocycles and their metal complexes will be explored using HPLC to improve the literature procedures. The amide precursors will also be studied in these assays to differentiate nucleophilic catalysis that could arise in the acids 6-8A.