PEPTIDES

PROCEEDINGS OF THE FIFTH AMERICAN PEPTIDE SYMPOSIUM

Murray Goodman

Johannes Meienhofer

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PREFACE

The Fifth American Peptide Symposium was held on the campus of the University of California, San Diego, from June 20-24, 1977. Representatives from more than twenty nations participated, demonstrating once again the truly international nature of peptide research. There has been an enormous increase in the study of peptides which the Program Committee recognized by bringing speakers to the meeting who created a sense of freshness and timeliness. We were also keenly aware of the need to maintain a balanced program that has become typical of the Peptide Symposia. Therefore we invited leaders in the areas of synthesis, biology, structure and characterization to present their latest findings.

A substantial departure from the format of the preceding meetings was inaugurated with this symposium. We invited only 14 main lectures. All submitted papers were presented in poster sessions. We also included an Open Forum which was designed to bring progress reports of ongoing work to the attention of peptide chemists and biologists.

During this symposium, we also presented the first Alan E. Pierce Award in peptide and chromatographic methodology. The recipient was Professor Miklos Bodanszky who in his award address stressed the uniqueness of each peptide sequence. He demonstrated superbly how peptide chemists must face new and undiminished challenges with each novel structure in the synthesis of complex peptides.

A major biological area of emphasis in the symposium involved brain peptides. The structures of enkephalin, endorphins and other fragments of β-lipotropin were actively discussed. Three of the main lecturers and many poster presentations concentrated on the isolation, characterization, analog syntheses and mode of action of these fascinating molecules. There was by no means complete agreement on the findings reported. Dr. Guillemin, in the concluding remarks of his lecture, took note of this when he indicated that the field is growing rapidly and that a comprehensive picture has not yet evolved.

Our symposium also showed the remarkable advances being made in analytical methodology. Dr. Udenfriend clearly showed that, by use of appropriate labels (radioactive and fluorescent) and modern chromatography, peptide and protein chemistry can be successfully accomplished at the picomole level. These techniques, together with the advances in sequencing and mass spectral analysis, provide us with incredibly sensitive assays of structure and purity.

Conformational studies of peptides, polypeptides and proteins were an important component of the symposium. Four main lectures and numerous posters were devoted to this area of research. From the presentations it is clear that theoreticians and experimentalists are continually learning from each other's results. The former are improving the basis for their potential functions and other ingredients used to predict correct potential energy minima. The latter

are unraveling many peptide and protein structures by X-ray crystallography and studies in solution. Utilizing nuclear magnetic resonance, charge-transfer spectra and related measurements, experimentalists are obtaining results which can be used to deduce conformations of peptides in solution. The theoretical studies aid the experimentalists in selecting appropriate measurements needed to propose molecular structures, while the theoreticians are able to refine their calculations on the basis of known structures from X-ray or studies in solutions.

The area of synthesis was well represented. In addition to the Pierce Award address in which Miklos Bodanszky described many exciting challenges remaining for peptide chemists, three main lectures and numerous posters were devoted to descriptions of sophisticated techniques of synthesis including protection, coupling and deprotection. The preparation of important and complex natural peptides was described. In their lectures, Drs. Sakakibara and Merrifield, using classical and solid-phase approaches respectively, showed that constant assessment of side reactions and racemization is necessary to achieve the level of successful syntheses of the natural products and model systems currently of interest. To these important factors, Dr. Feurer added scale-up and other industrial considerations for large-scale peptide synthesis.

As chairman of this year's meeting, I want to take this opportunity to thank the members of the Program Committee who worked closely with me in designing the program and in selecting the submitted papers for poster presentation. A meeting of the size of the Fifth American Peptide Symposium requires substantial funds to be launched successfully. A list of sponsors is contained elsewhere in these proceedings. I want at this point to thank each of the companies, and the Institute of General Medical Sciences of the National Institutes of Health for their generous financial support. There is no way that I can repay my secretaries, Charlotte Beck and Martha Parga, for their unstinting work. My colleague and associate, Dr. Michael Verlander, deserves much praise for his efforts in making sure that everything at the meeting went off according to schedule and without fuss. Much thanks should also go to members of my research group and the Local Committee who helped. Nomi Feldman, as coordinator of the operational aspects of the symposium, performed marvellously and beyond the call of duty. I especially want to acknowledge the efforts and professional insight of my editorial associate, Constance Mullin. To these people and all the attendees I owe a debt of gratitude.

Murray Goodman
La Jolla, California

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Alan E. Pierce Award Lecture

PEPTIDE SYNTHESIS: AN UNDIMINISHED CHALLENGE

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I would like to express my thanks to the Selection Committee of the American Peptide Symposia for choosing me as the first recipient of the Alan Pierce Award. However, no less gratitude is due to Mr. Roy Oliver of the Pierce Chemical Company. Peptide chemists have received, until now, no real appreciation such as the Pierce Award. This award is a timely recognition of the field of peptide chemistry, a field that requires arduous work, and unrelenting dedication. Last, but not least, I thank our Chairman and host, Professor Murray Goodman, for the invitation that allows a veteran of a quarter century to talk to you on peptide synthesis.

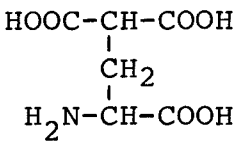
Time restrictions prevent a full presentation of recent advances, and I can mention only a few contributions. These are not necessarily the most significant ones: importance emerges gradually and can be judged only in retrospect. In fact, looking at the impressive array of well-established methods of protection, coupling, etc., one could even question the need for additional procedures. The answer is: yes, we need them, and the reasons for this affirmative answer can be found in the words of Theodor Wieland:[1]

"To the uninitiated, even to the chemist familiar with peptide chemistry only through hearsay, it may appear that nowadays peptide synthesis is mere routine. However, the adept has learned by experience that the individuality of the amino acids, which in the last analysis is one of the elements determining biological specificity, lends to their manipulation in synthesis such diversion, suspense and drudgery as found with no other substances. Thus only can the manifold efforts for development of a methodology of the field be understood. In progressing toward longer and longer, structurally well-defined peptide chains, methods, that are quite trustworthy with small peptides, may prove of little utility. Therefore, it should be possible to choose the right alternative from a large array of procedures. For all these reasons, every synthesis of a complicated polypeptide represents the sum of prolonged intellectual and experimental exertions which, however, often find their reward in the interesting physical, chemical and biological properties of the final product."

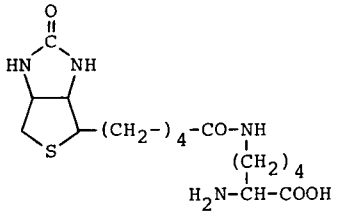
Unusual Residues

In addition to the twenty amino acids commonly found in proteins, many unusual residues were identified in microbial peptides, some of these requiring special measures of synthesis. We have to think only of the extreme readiness of phenylglycine to suffer racemization when activated.[2] Not less problematic

could be the incorporation of the newly recognized constituent of prothrombin, γ-carboxyglutamic acid.[3] The synthesis, resolution and protection of this amino acid (*1*) has already received growing attention.[4,5] In our own recent work, the incorporation of biocytin[6] (ε-biotinyl-L-lysine), (*2*) presented some problems.

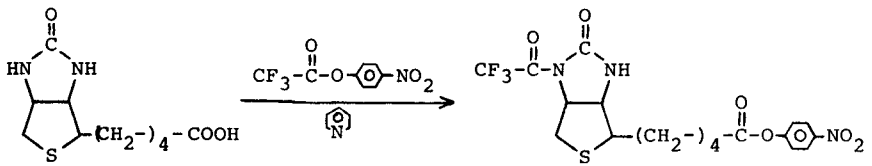


(*1*)



(*2*)

The attempted preparation of biotin *p*-nitrophenyl ester (*5*),[7] with the aid of *p*-nitrophenyl trifluoroacetate,[8] led to a trifluoroacetyl derivative (*4*),[9] which, in turn, could be converted to the desired active ester by alcoholysis. The weak, but definite, nucleophilic character of the urea grouping in biotin (*3*) did not prevent us[10] from obtaining a crystalline pentachlorophenyl ester of Boc-biocytin. [mp. 156° dec., $[\alpha]_D^{25}$ + 7° (*c* 2, DMF)]. Still, the complicating effect of biotin must be taken into consideration during our effort toward the synthesis of a biologically active tryptic fragment of the biotin-containing carboxyl carrier subunit of the transcarboxylase from *Propionibacterium shermanii.*[11]



(*3*)

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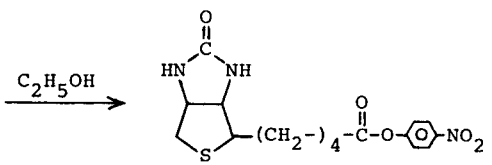
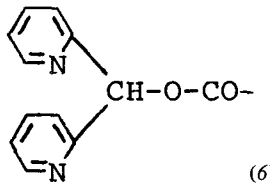


(*5*)

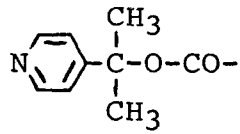
Unexpected behavior of an unusual residue occurred in the synthesis of a hormone analog in our laboratory.[12] Replacement of the side-chain amino group of the lysine residue in vasopressin by hydroxyl required the incorporation of ϵ-hydroxynorleucine. When the hydroxy group was left unprotected, the removal of α-amino protecting groups with hydrobromic acid in acetic acid or with trifluoroacetic acid caused considerable cleavage of the peptide bond between ϵ-hydroxynorleucine and the next amino acid, glycine, although the formation of a seven-membered lactone was the driving force of the N→O shift, not a five-membered one, as in the case of homoserine. An old device, acetylation of the hydroxy group, eliminated the problem, but it is obvious that unusual residues in naturally occurring peptides or in their analogs provide new stimuli for the development of novel protecting groups.

Protection and the Removal of Protecting Groups

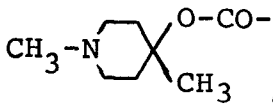
The introduction of tertiary amine substituents into well-established protecting groups renders them resistant to acids. This idea was generalized in Young's laboratory[13] in Oxford (groups *6–8*), though the underlying principle can be recognized in the earlier proposed[14] isonicotinyloxycarbonyl group (*9*) as well. The isonicotinyloxycarbonyl protection of the ϵ-amino group of lysine residues has already gained practical significance.



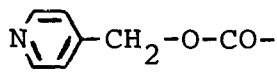
(*6*)



(*7*)

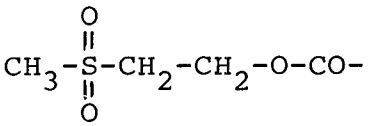


(*8*)



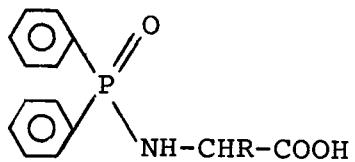
(*9*)

From the attempts toward acid-stable and selectively removable protection of the lysine side chain, the β-methylsulfonylethoxycarbonyl (Msoc) group (*10*) of Tesser[15] is quite promising. The alkali-catalyzed elimination reaction used for the removal of this group is remarkable in its smoothness, but the potential harm to other functions caused even by brief treatment with the calculated amount of



(10)

alkali still requires careful examination. An alternative solution for the stabilization of the benzyloxycarbonyl group against partial removal is substitution of the ring with negative atoms such as Cl[16] or Br[17] or electron-withdrawing groups (NO_2).[18] Such substituted Z-groups remain intact during acidolysis of Boc groups, e.g. with trifluoroacetic acid. On the other hand, unless hydrogenolysis can be used, the negatively substituted Z groups require quite strong acids for their removal. There is a general trend – coming from solid-phase peptide synthesis – for "global deprotection," that is, for the simultaneous removal of all protecting groups with strong acidic reagents. The application of HF,[19] BBr_3,[20] $B(CF_3COO)_3$,[21] CF_3SO_3H,[22] and CH_3SO_3H[23] are all steps in this same direction, as is the potential use of the HF-pyridine complex.[24] Yet, such strong acidic conditions are conducive to previously unobserved side reactions, e.g. the migration of benzyl groups in tyrosine from oxygen into the nucleus. Therefore, some new consideration is due to the "old faithful," the Z-group.[25] Instead of rendering it more acid resistant, one can change the conditions of the cleavage of the Boc group in such a way that the Z-groups on lysine side chains remain essentially intact. Dilution of trifluoroacetic acid with 30% water was proposed by Schnabel et al.,[26] but concern about hydrolysis led us[27] to use a mixture of 70% trifluoroacetic acid and 30% acetic acid, which provides complete removal of Boc groups in about 1/2 hour at room temperature, yet causes only negligible cleavage of side chain Z-groups. A sophisticated approach, reaching selectivity with weaker rather than stronger acids, was proposed by the Ciba-Geigy group.[28] The trityl and biphenylisopropyloxycarbonyl (Bpoc) groups can be selectively removed, each at a distinct, well-defined acidity in 90% trifluoroethanol. The new method found impressive application in a most elegant synthesis of human insulin.[29] An interesting new class of acid labile amino-protecting groups, phosphinamides, was proposed by Kenner et al.[30] Protection of amino acids in the form of diphenylphosphinamides (*11*) protects them against racemization as well.



(11)

A similar pattern of development can be recognized in connection with the protection of the phenolic hydroxyl of tyrosine. The "classical" protecting group, *O*-benzyl (*12*),[31] was used without complication, when HBr in AcOH[32]