



INTERNATIONAL JOURNAL OF

# Peptide & Protein Research

Head office:  
MUNKSGAARD International Publishers Ltd.  
35 Nørre Søgade  
Postbox 2148  
DK-1016 Copenhagen K, Denmark

or  
Regional office in USA:  
MUNKSGAARD International Publishers Ltd.  
Three Cambridge Center  
Suite 208  
Cambridge, MA 02142  
USA

or with any bookseller.

## Aim and Scope

The INTERNATIONAL JOURNAL OF PEPTIDE & PROTEIN RESEARCH will be of the highest possible scientific and technical standard and will cover not only proteins as such but also peptides and amino acids. The Journal will be open to original papers that contribute to the further development of peptide and protein research.

## Rapid Publication

This is indeed a large field and there is need for a journal that can publish the latest results in the shortest possible time. It is, therefore, a principal aim to keep publication time at an absolute minimum, ensuring that articles within the field of peptide and protein research are gathered quickly in one place and thereby are of easy access to all interested in the field. Special attention will be given to the rapid publication of Short Communications.

## Subscription 1991

Two volumes are published annually, one issue per month. Subscription price 1991: DKK 2700.00 postage included (GBP 253.00, DEM 750.00). USA, Canada and Japan 1991: USD 513.00 including postage and air freight.

**Reduced rate for private subscribers 1991:** DKK 1460.00 postage included (GBP 137.00, DEM 405.00). USA, Canada and Japan: USD 287.00 including postage and air freight.

Prices are subject to exchange-rate fluctuations.

© 1991 Munksgaard International Publishers Ltd. Authorization to photocopy items for internal or personal use, or the internal or personal use of specific clients, is granted by Munksgaard International Publishers, Ltd. for libraries and other users registered with the Copyright Clearance Center (CCC) Transactional Reporting Service, provided that the base fee of \$02.50 per copy is paid directly to CCC, 27 Congress Street, Salem, MA 01970. 0367-8377/91/\$02.50 + 0.00. All other rights, including microfilm, reserved.

*International Journal of Peptide & Protein Research* (ISSN 0367-8377) is published monthly by Munksgaard International Publishers Ltd, 35 Nørre Søgade, P.O. Box 2148, DK-1016 Copenhagen K, Denmark. USA subscription price is USD 513.00 including airspeed delivery. Second class postage paid at Jamaica, NY 11431. USA Postmaster for North American subscribers: send address changes to Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003. Air freight and mailing in the USA by Publications Expediting Inc. Printed in Great Britain at the Alden Press, Oxford.

## Editor-in-Chief

Victor J. Hruby, USA

## Editorial Board

R. Acher, France  
P. Balam, India  
E. Benedetti, Italy  
N. L. Benoiton, Canada  
E. Breslow, USA  
B. Castro, France  
C. Deber, Canada  
W. DeGrado, USA  
R. E. Feeney, USA  
L. Gierasch, USA  
N. Gō, Japan  
M. Goodman, USA  
R. Hirschmann, USA  
V. T. Ivanov, USSR  
J. H. Jones, England  
G. Jung, Germany  
I. Karle, USA  
D. S. Kemp, USA  
H. Kessler, Germany  
K. D. Kopple, USA  
M. Lebl, Czechoslovakia  
M. Marraud, France  
G. Marshall, USA  
R. B. Merrifield, USA  
D. H. Rich, USA  
R. Rocchi, Italy  
B. P. Roques, France  
S. Sakakibara, Japan  
H. A. Scheraga, USA  
P. W. Schiller, Canada  
R. C. Sheppard, England  
J. A. Smith, USA  
A. F. Spatola, USA  
G. I. Tesser, The Netherlands  
G. Van Binst, Belgium  
H. Yajima, Japan

This material was copied  
at the NLM and may be

## Original Articles

Determination of the disulfide bond pairings in bovine transforming growth factor- $\alpha$	463	B.N. Violand, J.S. Tou, B.D. Vineyard, N.R. Siegel, C.E. Smith, P.D. Pyla, J.F. Zobel, P.C. Toren & E.W. Kolodziej
Preparations, solution conformations and molecular structures of <i>N,N'</i> -ethylene-bridged dipeptides and their derivatives	468	Y. Kojima, Y. Ikeda, E. Kumata, J. Maruo, A. Okamoto, K. Hirotsu, K. Shibata & A. Ohsuka
Synthesis of S-alkyl and C-terminal analogs of the <i>Saccharomyces cerevisiae</i> a-factor. Influence of temperature on the stability of Fmoc and OFm groups toward HF	476	C-B. Xue, J.M. Becker & F. Naider
General method for rapid synthesis of multicomponent peptide mixtures	487	A. Furka, F. Sebestyén, M. Asgedom & G. Dibó
Hydrolysis of $\beta$ -casein by gastric proteases. I. Comparison of proteolytic action of bovine chymosin and pepsin A	494	H. Guillou, G. Miranda & J-P. Pelissier
Cyclization studies with tetra- and pentapeptide sequences corresponding to $\beta$ -casomorphins	502	R. Schmidt & K. Neubert
Infrared spectroscopic discrimination between $\alpha$ - and $3_{10}$ -helices in globular proteins. Reexamination of Amide I infrared bands of $\alpha$ -lactalbumin and their assignment to secondary structures	508	S.J. Prestrelski, D.M. Byler & M.P. Thompson
2-Chlorotriptyl chloride resin. Studies on anchoring of Fmoc-amino acids and peptide cleavage	513	K. Barlos, O. Chatzi, D. Gatos & G. Stavropoulos
Peptides from chiral C <sup>2,2</sup> -disubstituted glycines. Crystallographic characterization of conformation of C <sup>2</sup> -methyl, C <sup>2</sup> -isopropylglycine [( $\alpha$ Me)Val] in simple derivatives and model peptides	521	G. Valle, M. Crisma, C. Toniolo, S. Polinelli, W.H.J. Boesten, H.E. Schoemaker, E.M. Meijer & J. Kamphuis
Conformations of neurotensin in solution and in membrane environments studied by 2-D NMR spectroscopy	528	G-Y. Xu & C.M. Deber
Conformation and inhibitory properties of peptides based on the tissue kallikrein-angiotensin complex	536	M.S. Deshpande, J. Boylan, J.A. Hamilton & J. Burton
Amatoxins bearing amino and carboxyl groups prepared by selective alteration of the aldehyde generated by periodate oxidation of methylated $\alpha$ -amanitin	544	J.E. Mullersman & J.F. Preston, III
Synthesis and biological evaluation of mouse growth hormone-releasing factor	552	E.P. Heimer, M. Ahmad, T.J. Lambros, A.M. Felix, T.R. Downs & L.A. Frohman
Enhancement of solubility by temporary dimethoxybenzyl-substitution of peptide bonds. Towards the synthesis of defined oligomers of alanine and of lysyl-glutamyl-glycine	556	J. Blaakmeer, T. Tijssse-Klasen & G.I. Tesser
Evidence for a glycoconjugate form of glutathione S-transferase pI	565	S. Kuzmich, L.A. Vanderveer & K.D. Tew
Fmoc/solid-phase synthesis of Tyr( <i>P</i> )-containing peptides through <i>t</i> -butyl phosphate protection	572	J.W. Perich & E.C. Reynolds

29310  
220

Munksgaard · Copenhagen

This material was copied  
from the NLM and may be

## General method for rapid synthesis of multicomponent peptide mixtures

ÁRPÁD FURKA, FERENC SEBESTYÉN, MAMO ASGEDOM\* and GÁBOR DIBÓ

*Department of Organic Chemistry, Eötvös Loránd University, Budapest, Hungary*

Received 12 February, accepted for publication 21 November 1990

A method is suggested for the synthesis of multicomponent peptide mixtures. The method is a solid phase synthesis modified in order to give a closely equimolar mixture of peptides with predetermined sequences. The main point of modification is that before every coupling cycle the resin is divided into equal parts and each portion is coupled with a different amino acid. Then the portions are mixed and before the next coupling cycle the resin is again distributed into equal portions. The method is illustrated by the synthesis of a mixture of 27 tetrapeptides and that of 180 pentapeptides.

*Key words:* peptide mixtures, synthetic; peptide synthesis, new method; peptide synthesis, solid phase; peptides, electrophoretic identification; peptides, HPLC separation

Due to the outstanding importance of peptides in biological processes there is an increasing need for synthetic peptides in a variety of applications. Although the introduction of the solid phase method (1) and its automation have considerably speeded up the synthetic procedure itself, the one by one synthesis of peptides still seems to be slow to comply with the need. A possible strategy to improve the productivity of the synthetic methods is the simultaneous synthesis of two or more—even several hundreds of—peptides. Different methods have been developed to achieve this goal. van Rietschoten *et al.* (2) succeeded in synthesizing two peptides on two easily separable resins. Gorman (3) constructed a multi-vessel apparatus and successfully applied it for the simultaneous synthesis of four peptides. The multiple continuous-flow solid phase method devised by Krchnak *et al.* (4) made possible the synthesis of a decapeptide and its nine omission analogs in a single run. Geysen *et al.* (5) synthesized 208 hexapeptides on polyethylene rods and tested them without removal from the solid support. In the remarkable method of Houghten (6) 40–80 peptides were simultaneously synthesized on 40–80 portions of resin placed in solvent-permeable

bags. Frank & Döring (7) applied paper discs as solid support in their synthesis and the coupling operations were carried out on 100 discs at a time. Much labor can be saved by using simultaneous synthetic methods, which is well demonstrated by Houghten's experiments in which 260 different 13-residue peptides were synthesized in less than 4 weeks.

Further improvement can be expected in the efficiency of the synthesis if one compromises by using peptide mixtures instead of individual peptides. Tjoeng *et al.* (8) succeeded in synthesizing mixtures of four to seven oligopeptides in a single run. The mixture was synthesized on solid support by using, in one of the coupling cycles, a mixture of four to seven acylating amino acid derivatives.

By exploiting the additional possibilities inherent in the Merrifield method, a new synthetic strategy can be introduced assuring, besides a dramatic reduction in the number of the coupling cycles, the closely equimolar formation of the components of the peptide mixture (9).

*The principle of the method.* A mixture of a large number of peptides, each of them containing the same number of residues but different sequences (which can be deduced from that of a parent peptide by varying amino acids in all or several positions), can be synthesized in a single run. A normal solid phase synthesis is carried out, but before every coupling step the resin is

Abbreviations follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (*European J. Biochem.* 138, 1984, 9–37).

\*Present address: P.O. Box 62379, Addis Ababa, Ethiopia.

divided into equal parts (the number depending on the number of amino acids intended to vary in that particular position). Each portion is coupled with the desired amino acid, and the samples are then mixed. The number of the components in the mixture synthesized this way is given by the product of the numbers of the amino acids varied in the different positions.

#### MATERIALS AND METHODS

Reagents and solvents were products of Fluka AG (Buchs, Switzerland). Boc-amino acids were purchased from Reanal (Budapest, Hungary). Side chain protecting groups were: OBzl for Glu and Z for Lys. Boc-Ala-resin (0.74 mmol Ala/g) was prepared from Bio-Beads S-X1 chloromethyl resin (Bio-Rad Laboratories, Richmond, CA) by Gisin's method (10).

*Solid phase synthesis.* The coupling protocol of Gutte & Merrifield (11) was adapted with slight modifications: diisopropylcarbodiimide (12) and 1-hydroxybenzotriazole (13) were used; the Boc-amino acids were added in 100% molar excess; the activated Boc-amino acid derivatives were dissolved in dichloromethane-dimethyl formamide mixture (3:1, v/v). The progress of the coupling reaction was followed with Kaiser's ninhydrin test (14).

*Mixing and portioning of the resin samples* were performed on Boc-protected peptidyl resins. The different resin samples (ca. 120 mg of each) were suspended in 10–10 mL of dimethylformamide and poured into a common vessel. The mixture was shaken for 10 min and, to avoid sedimentation, quickly divided into equal volumes.

*Cleavage of peptides* from the resin (50 mg) was carried out by trifluoromethanesulfonic acid (15). The reaction mixture was filtered and washed with trifluoroacetic acid into 25 mL dry ether. The mixture was allowed to stand overnight at  $-20^{\circ}\text{C}$ , then the precipitate was collected by filtration, washed twice with ether and dried over KOH, then over  $\text{P}_2\text{O}_5$ .

*HPLC separation of the peptides* was performed on a Vydac 218TP54 C18 (25 cm  $\times$  2.1 mm, i.d.) reversed-phase column using a Beckman system (Model 421 Controller, Model 340 Organizer). Elution was isocratic at 100% A (0.1% aqueous trifluoroacetic acid) for 5 min; then followed by a linear gradient of 0–20% B (90% acetonitrile containing 0.1% trifluoroacetic acid) for 20 min, and 20–50% for an additional 20 min. The flow rate was 1 mL/min. The peptides were detected at 214 nm (0.5 AUFS).

*Sequential degradation* was carried out on a gas-phase sequencer built at the City of Hope according to the method of Hawke *et al.* (16) using the continuous-flow

reactor of Shively *et al.* (17); the phenylthiohydantoin amino acids were identified by using an on-line reversed-phase HPLC system.

*Two-dimensional paper electrophoresis.* The samples of the peptide mixtures were applied to a 30 cm band on Whatman 3 MM paper (0.3–0.4 mg/cm), together with reference markers of taurine and leucine methyl ester (50 nmol/cm) on both sides. Electrophoresis was accomplished on a horizontal cooled plate apparatus (Labor MIM, Hungary) at 32 V/cm. The first run was made at pH 6.5 for 2 h, using pyridine acetate buffer (18). After drying, two side strips were cut out and stained with cadmium-ninhydrin (19). A guide strip of 2.5 cm (parallel to the side strips) was excised and sewn to a fresh sheet. Electrophoresis was performed, again with markers on the two sides, in a perpendicular direction at pH 2.0 applying AcOH-formic acid solution as buffer (20). The peptide map was stained and the migration distances then measured.

For the preparation of the individual peptides, the bands were cut out from the first electrophoretogram, stitched to new sheets. Each sheet was subjected to electrophoresis at pH 2.0 for 2 h. The ninhydrin-positive strips were cut out and the peptides were eluted from the paper with dilute AcOH, then freeze dried.

#### RESULTS

*Synthesis of a mixture of 27 tetrapeptides.* The components of the mixture to be synthesized were designed to have Ala at the C-terminus and, in the remaining 3 positions, Glu, Phe and Lys were varied.

TABLE I  
Peptides formed on polymer (p) support as result of the three coupling steps in synthesis of 27 tetrapeptides

A-p		
	Coupling step 1	
EA-p	FA-p	KA-p
	Coupling step 2	
E EA-p	FEA-p	KEA-p
E FA-p	FFA-p	KFA-p
E KA-p	FKA-p	KKA-p
	Coupling step 3	
E E EA-p	FEEA-p	KEEA-p
E E FA-p	FEFA-p	KEFA-p
E E KA-p	FEKA-p	KEKA-p
E FE A-p	FFEA-p	KFEA-p
E FF A-p	FFFA-p	KFFA-p
E FK A-p	FFKA-p	KFKA-p
E KE A-p	FKEA-p	KKEA-p
E KF A-p	FKFA-p	KKFA-p
E KK A-p	FKKA-p	KKKA-p

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

## LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

## FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

## E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.