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NEW REAGENTS FOR CARBOXYL-TERMINAL PEPTIDE SEQUENCING

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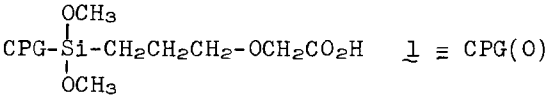
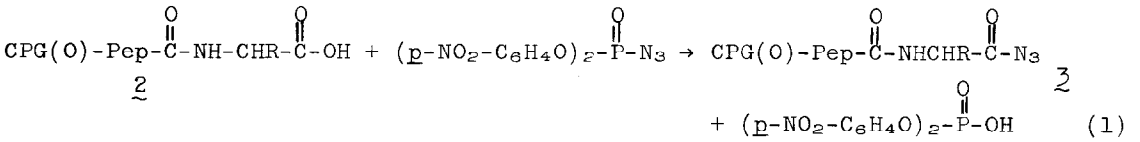
The ready availability of solid supports of various types has opened the peptide field to a wide variety of reagents. Our program has been aimed at, among other things, the development of a carboxyl-terminal sequential peptide degradation. Ironically, the earliest efforts directed toward the development of a carboxyl-terminal degradation[2-4] antedate the highly successful Edman amino-terminal procedure[5] by several years. Despite the success of the Edman method, a method for the sequential degradation of peptides from the carboxyl terminus would complement the Edman procedure and would be highly useful in a number of situations[6]. Our initial efforts in the field have led to the development of a solution procedure for the removal of the carboxyl-terminal amino acid residue[7,8]; we here report our initial results in the development of a sequential process using solid phase methodology and two interesting reagents.

Peptides were insolubilized on a modified Controlled Pore Glass (CPG) solid support,[9] 1, by first forming the N-hydroxysuccinimide ester of the glass derivative with N-hydroxysuccinimide-O-trifluoroacetate[10] in dry

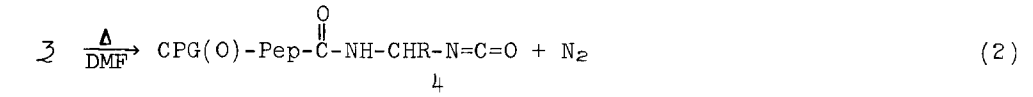


1 ≡ CPG(O)

pyridine and reaction of the ester with an aqueous solution of the peptide at pH 7.25 in an aqueous solution which was 8M in urea. This procedure attaches peptides to the glass specifically by their amino terminus. The resulting CPG(O)-peptide was heated at 70° in a 0.2M $NaHCO_3$ buffer, pH 8.5, for 5 min, and washed with water and dimethylformamide (DMF). The insolubilized peptide was then treated with bis(p-nitrophenyl)phosphoryl azide[12] in "buffered DMF"[13] for 10 min at room temperature. The solution was made 25 mol % in p-methoxybenzyl alcohol and heated at 70° for 1 hr. Normally, this procedure was repeated twice, and the results were analyzed by difference amino acid analysis. This procedure effects the following transformations (eq 1-3).



(1)



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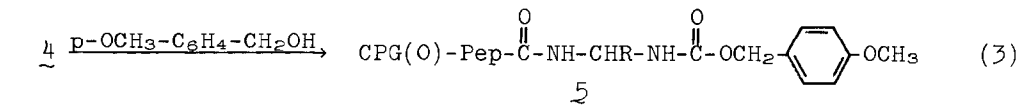
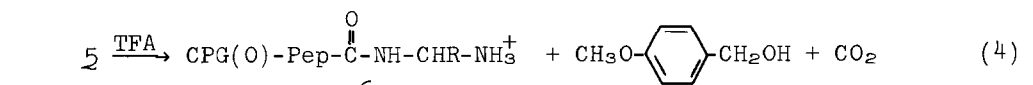


(3)

The usual 21 hr/110°/6N HCl conditions carried out directly on a sample of the glass hydrolyze 5 to its constituent amino acids; comparison of the amino acid analysis of this sample to that of 2 shows the carboxyl-terminal amino acid to be missing (Table 1).

Continuation of a sequential degradation is realized by treatment of 5 with anhydrous trifluoroacetic acid (TFA) to yield the interesting derivative 6, which is itself further transformed to 7 by heating in buffered water for 80 min. Derivative 7, which is the amide of the amino acid penultimate to the carboxyl-terminal residue of the starting peptide 2, undergoes a Hofmann-type rearrangement under the influence of I,I-bis(trifluoroacetoxy)iodobenzene[14] in TFA/water/DMF to yield directly 8, exactly analogous to 7, but one amino acid residue shorter. This latter procedure can then be repeated sequentially



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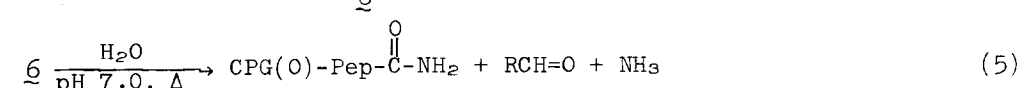

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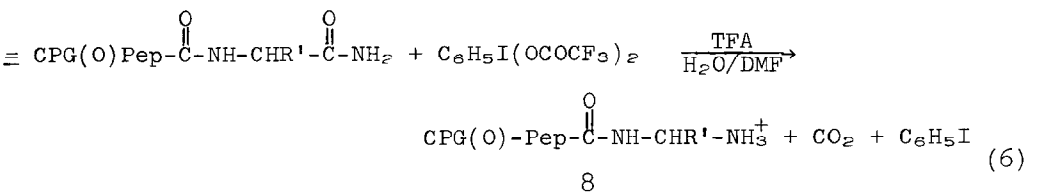

(6)

The results of a sequential procedure applied to a six-residue peptide amide are presented in Table 2. The sequence of this peptide is readily determined from the data in the Table as Lys·Phe·Ile·Gly·Leu·Met(amide).

That no unforeseen complications attend the use of the two procedures sequentially on the same peptide is exemplified by the application of both types of degradative methodology to the peptide Gly·Leu·Ala (eq 7).

Table 1. Results of the Procedure to Remove the Carboxyl-Terminal Residue of Peptides with Bis(p-nitrophenyl)phosphoryl Azide

No.	CPG-Peptide[a,b]	Degraded CPG-Peptide[c]	% Degradation
1	Gly(1.0)Asp(0.95)	Gly(1.0)Asp(0.06)	94
2	Ala(1.0)Ser(0.93)	Ala(1.0)Ser(0.09)	91
3	Gly(1.0)Glu(0.93)	Gly(1.0)Glu(0.0)	100
4	Ala(1.0)Pro(1.0)	Ala(1.0)Pro(0.05)	95
5	Ala(1.0)Leu(0.97)Gly(0.98)	Ala(1.0)Leu(0.99)Gly(0.05)	99
6	Phe(1.0)Asp(0.97)Ala(0.97) Ser(0.86)Val(0.97)	Phe(1.0)Asp(0.50)DAPA(0.42)[d] Ala(0.99)Ser(0.80)Val(0)	100
7	Ala(1.0)Ile(1.04)	Ala(1.0)Ile(0.01)	99
8	Gly(1.0)Met(0.78)	Gly(1.0)Met(0.09)	69-91[e]
9	Gly(1.0)Leu(0.85)Tyr(0.84)	Gly(1.0)Leu(1.01)Tyr(0.08)	76-92[e]
10	Glu(0.97)Gly(1.0)Phe(1.01)	Glu(0.15)DABA(0.60)[d]Gly(1.0) Phe(0)	100
11	Pro(0.95)Phe(1.0)Gly(0.96) Lys[ε-acetyl](0.92)	Pro(1.0)Phe(1.01)Gly(1.0) Lys(0.03)	97
12	Gly(1.0)His(0.98)	Gly(1.0)His(0.01)	98
13	Thr(1.12)Lys(1.0)Pro(1.13) Arg(1.04)	Thr(0.93)Lys(1.0)Pro(1.1) Arg(0.08)	92
14	Bradykinin [Ser(0.96)Pro (2.94)Gly(1.00)Phe(2.06) Arg(1.82)] (C-terminal Arg)	Ser(0.92)Pro(2.40)Gly(1.00) Phe(1.93)Arg(0.88)	100
15	Insulin A-Chain (C-terminal Asn)	Loss of 0.90 Asn (as Asp)	90

[a]Amino acid analysis prior to degradation. [b]Glass support used was an aminopropyl-CPG which proved to be less satisfactory than CPG(0) in subsequent operations; CPG(0) was shown to be as good in this procedure in several experiments. [c]Amino acid analysis after degradation. [d]DAPA = 2,3-diaminopropionic acid; DABA = 2,4-diaminobutyric acid; these are the products expected from the rearrangement of the side chain carboxyl groups of Asp and Glu, respectively. [e]Uncertainty in yield reflects uncertainty in original amino acid analysis.

Table 2. Sequential Degradation of Eledoisin-related Peptide Amide from the Carboxyl Terminus with I,I-Bis(trifluoroacetoxy)iodobenzene[a]

Residue	Amino acid analysis after cycle 0	1	2	3	4	5
Gly	0.99	1.00	0.90	0.37	0.39	0.40
Met	0.66	0.00	0.00	0.00	0.00	0.00
Ile	0.99	1.00	1.05	0.82	0.47	0.43
Leu	1.00	0.88	0.29	0.20	0.19	0.20
Phe	1.00	1.00	1.01	0.99	0.88	0.70
Lys	1.00	1.00	1.00	1.00	1.00	1.00

Residue→ / Cycle↓	Met	Leu	Gly	Ile	Phe	Lys
1	-100%	-	-	-	-	-
2	-100%	-71%	-	-	-	-
3	-100%	-80%	-63%	-	-	-
4	-100%	-81%	-61%	-53%	-	-
5	-100%	-80%	-60%	-57%	-30%	-

[a]Left side of Table gives actual amino acid analysis results for each cycle of degradation; right side gives the per cent degradation of each amino acid residue as a function of cycle number.

$$\text{CPG(O)Gly(1.0)Leu(0.99)Ala(0.95)} \xrightarrow[\text{phosphoryl azide}]{\text{bis(p-nitrophenyl)-}} \xrightarrow{\text{TFA}} \xrightarrow[100^\circ]{H_2O,\ pH\ 7.0} \text{CPG(O)-Gly(1.0)Leu(0.98)Ala(0.02)} \quad [98\%]$$

$$2 \xrightarrow[TFA/H_2O/DMF]{C_6H_5I(OCOCF_3)_2} 2\,\text{CPG(O)Gly(1.0)Leu(0.12)Ala(0.03)} \quad [88\%;\ 90\%\ \text{of available Leu}] \qquad (7)$$

The interesting reagent diphenylphosphoryl azide was proposed by the Yamada group[12,15] for the direct racemization-free synthesis of acyl azides. The dinitro derivative used here brings about azide formation with an enhanced rate, but significant racemization accompanies the reaction. This presents no problem in our procedure, however, since the asymmetric carbon of the terminal amino acid is liberated as a trigonal center. The repetitive yield in the sequential degradation is about 80%, roughly equivalent to that in the "manual" Edman degradation. We have some indication that the remaining 20% not lost has been terminated by an I,I-bis(trifluoroacetoxy)iodobenzene-promoted reaction of functional groups on the solid support with some degradation intermediate, perhaps the isocyanate. Whatever this reaction is, it effectively terminates the degradation when it occurs, since further losses are not observed once a residue has been presented to the reagent as a C-terminal amide. The results reported here have encouraged us to continue our program by investigating in detail the effects of functionalized side chains and direct identification of the aldehyde fragment produced in the degradation.

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