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Dithioketal Formation During Synthesis of Bpa Containing Peptides.

Michael Breslav,[a] Jeffrey Becker,[b] and Fred Naider.*[a]

[a]Department of Chemistry, College of Staten Island, City University of New York, Staten Island, New York 10314.

[b]Departments of Microbiology and Biochemistry, Cellular and Molecular Biology, University of Tennessee, Knoxville, Tennessee 37996.

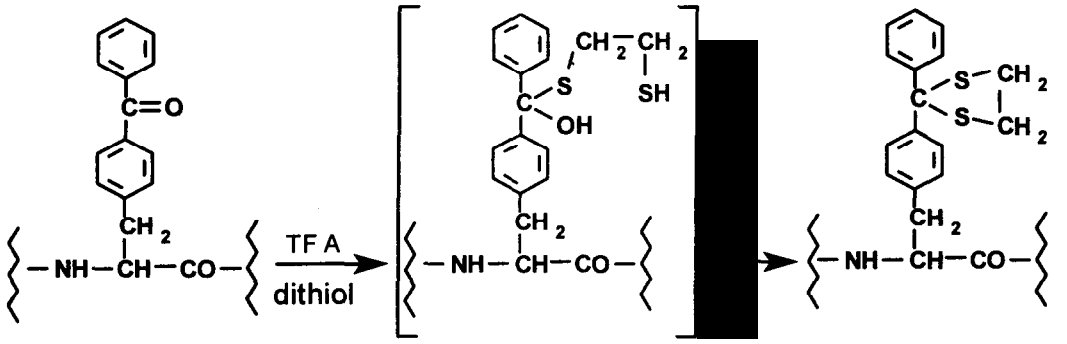
Abstract. Acidolytic cleavage of peptides from polymeric supports often employs dithiols as scavengers. A significant impurity was found during the synthesis of peptides containing *p*-benzoylphenylalanine (Bpa) when 1,2-ethanedithiol was the scavenger. Herein we describe a new side reaction involving Bpa containing peptides and dithiols that results in dithioketal formation and report a strategy to eliminate this side reaction. © 1997 Elsevier Science Ltd.

p-Benzoylphenylalanine (Bpa) is extensively employed in studies attempting to define the binding site of peptide hormones to receptors. Incorporation of this amino acid derivative into biologically active peptides, followed by photolysis in the presence of the receptor results in a high energy intermediate that inserts into C - H bonds of the receptor.[1] Thus, a covalent bond is created between the receptor and the peptide which on further investigation can define both the region of the receptor involved in binding and specific residues which contact the peptide hormone.

We are attempting to prepare Bpa scanned analogs of the *Saccharomyces cerevisiae* α-factor - a tridecapeptide which is recognized by a 7-transmembrane G protein coupled receptor.[2,3] Two of these analogs carrying Bpa in positions 1 or 3 of the α-factor sequence were prepared previously using Boc chemistry and manual solid phase peptide synthesis on a PAM resin followed by HF cleavage.[4] Recently peptides bearing Bpa in different positions were synthesized automatically (433A Peptide synthesizer, Applied Biosystems) using conventional Fmoc based chemistry on a solid phase support (HMP resin).[5] After each analog was assembled on the resin, the peptide was released using a standard cleavage protocol which included treatment of the peptide-resin with a mixture of TFA/H_2O/EDT (95/2.5/2.5 v/v) for 1.5 hours at room temperature. [6]

All crude peptides obtained using these conventional conditions exhibited 2 major components in ratios from 1:1.2 to 1:8 on HPLC analysis.[7] Both major products from each synthesis were isolated using RPHPLC.

MS results and amino acid analysis for [Arg[7], Nle[12], Bpa[13]]α-factor demonstrated that the compound corresponding to the faster moving peak (Compound 1) was the desired analog.[8] The second slower moving peak (compound 2) exhibited a 76 Da increase in mass. Both major products contained all amino acid residues.[9] Sulfur analysis on compound 2[10] and the almost complete loss in the Bpa derived absorbance at 260 nm for this compound in comparison to compound 1 led us to conclude that the second product was the desired peptide sequence with a dithioketal in place of the diarylketone of Bpa (Scheme 1).[11]



Scheme 1. Transformation of Bpa-containing peptide to a cyclic dithioketal derivative.

Although side reaction of 1,2-ethane dithiol with Trp residues during acidolysis has been reported[12], side reactions for Bpa-containing peptides with thiols have not appeared in the literature. The protection of the carbonyl group by reaction with a thiol or dithiol in the presence of an acid catalyst has been described for small organic molecules.[13] In particular 1,3-dithiolanes are synthesized by acid-catalyzed condensation of carbonyl compounds with 1,2-ethanedithiol.[14]

Fmoc chemistry is now widely applied in peptide synthesis and represents an excellent strategy for preparation of Bpa-containing peptides. Therefore, we decided to examine the side reaction with dithiols in detail. To standardize this analysis we synthesized a model tripeptide Ac-Ala-Bpa-Leu using the Fmoc chemistry protocol and cleaved this peptide from the resin with EDT added to, or deleted from, the cleavage cocktail.[15] Without EDT only Ac-Ala-Bpa-Leu was formed, however the presence of EDT in the cleavage cocktail led to the nearly quantitative formation of the peptide bearing a cyclic dithioketal.[15]

Since thiols represent important scavengers for the prevention of alkylation by carbocations generated during acidolytic deprotection of peptides we tested several dithiols under similar conditions.[16] We performed our analysis at 5°C in order to make the reaction time convenient for monitoring. Kinetic analyses of the reaction of 1,2-ethanedithiol, 1,3-propanedithiol, 2,3-butanedithiol, dithiothreitol and 1,4-dithio-L-threitol with Ac-Ala-Bpa-Leu were conducted using automated HPLC monitoring [Fig. 1].

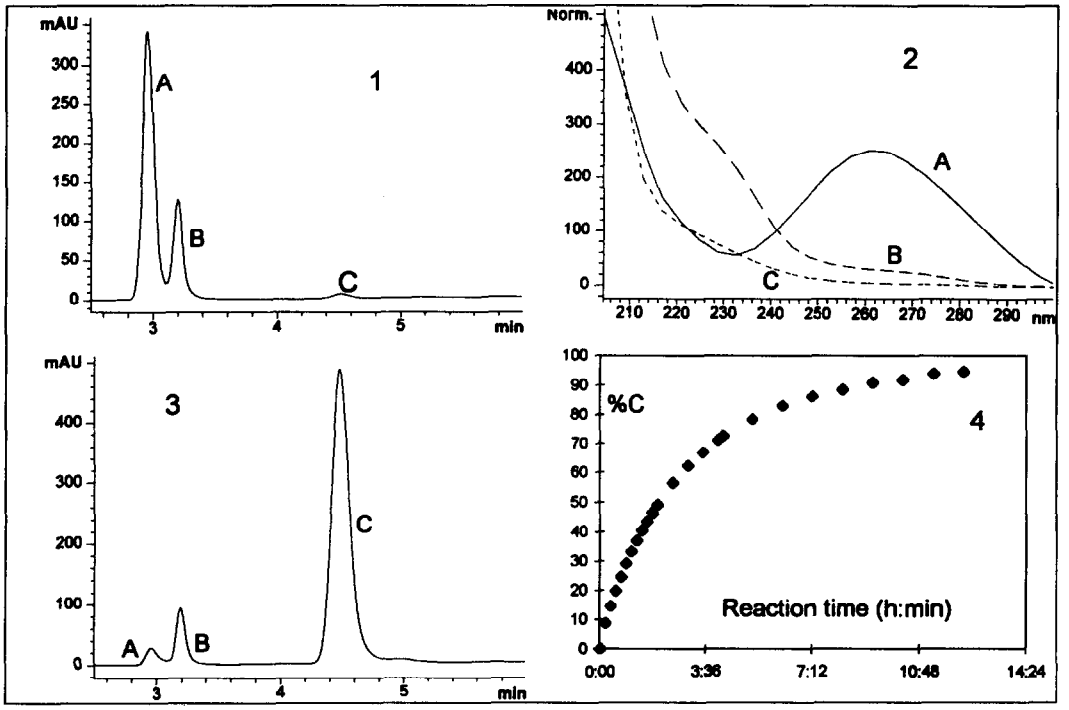


Fig. 1. Formation of 1,3-Dithiolane derivative from Ac-Ala-Bpa-Leu-OH at 5°C; A: Ac-Ala-Bpa-Leu-OH; B: hemimercaptal derivative; C: 1,3-Dithiolane derivative; Panel 1: HPLC: 2 minutes after start of the reaction; Panel 2: UV spectra ; Panel 3: HPLC: after 12 hours of reaction; Panel 4: % Thioketal formed at various times (% of A to C transformation).[17]

Table 1 The effect of dithiol on the rate of dithioketal formation.

Dithiol HS-R-SH	Time of 50% transformation A to C (h:min)	Retention ($k'=k-k_o/k_o$) of the dithioketal C	Retention of the hemimercaptal B
$-CH_2-CH_2-$	2:24	1.26	0.60
$-CH_2-CH_2-CH_2-$	3:20	1.60	0.82
$CH_3-\underset{\displaystyle OH}{CH}-\underset{\displaystyle OH}{CH}-CH_3$	6:15	2.24	1.18
$-CH_2-\underset{\displaystyle OH}{CH}-\underset{\displaystyle OH}{CH}-CH_2-$	No reaction.	-	-

The study indicated that the formation of the 5 membered dithioketal with EDT proceeded faster than formation of the 6-membered cycle with 1,3 propanedithiol [Table 1]. However, formation of the 5-membered cycle with 2,3-butanedithiol proceeded even more slowly indicating an influence of steric hindrance and/or decreased nucleophilicity to the rate of the dithioketal formation. No reaction was observed when dithiothreitol or 1,4-dithio-L-threitol were applied. These latter results indicate that dithiothreitol, which has been described previously as a possible scavenger [18] for Boc/HF chemistry is

safe to use with Bpa containing peptides. In addition we also noticed formation of the product having intermediate retention time [Fig. 1], which we believe is a hemimercaptal[19].

The presence of the Bpa moiety in a peptide renders this compound light sensitive. Special precautions must, therefore, be taken during synthesis and storage of Bpa-containing probes. The dithiolane derivative of *p*-benzoylphenylanaline could be used as a protecting group during peptide synthesis if the thioketal could be converted back to the desired diarylketone in peptides under mild conditions. We are presently working on regenerating BPA from its dithioketal derivative in peptides.

In conclusion we report herein the observation of a new side reaction during the synthesis of peptides containing *p*-benzoylphenylalanine. Cyclic dithioketals which are formed during acidolysis in the presence of dithiols can be eliminated by using dithiothreitol in place of ethane dithiol.

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References and Notes

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3. Sequence of the α-factor: WHWLQLKPGQPMY. Bpa scanning was applied to analog: WHWLQLRPGQP-Nle-Y.
4. Jiang, Y.; Breslav, M.; Khare, R.; McKinney, A.; Becker, J.; Naider, F., *Int. J.Pept. Prot. Res.,* **1995,** *45,* 106 - 115.
5. Synthetic details and biological activity data will be reported elsewhere.
6. Introduction to cleavage techniques; Manual; Applied Biosystems Inc.; **1990,** p. 10.
7. The peptide with a longer retention time was the major product. Chromatographic conditions for analytical HPLC: μ-Bondapack C_{18} HPLC column (3.9 x 300 mm), acetonitrile/water (0.025% TFA) gradient from 30 to 80%, flow rate 1.5 ml/min, diodarray detection employed.
8. Compound 1: ES-MS (m/z) Found: 1783.1; Calculated for $C_{90}\ H_{120}\ N_{22}\ O_{17}$ (MH^+) 1782.9. Amino acid analysis: Glx 2.12 (2); Pro 1.95 (2); Gly 0.99 (1); Leu 1.99 (2); Nle 0.95 (1); His 0.91 (1); Arg 0.97 (1). Bpa can not be determined.
9. Compound 2: ES-MS (m/z) Found: 1859.0; Calculated for $C_{92}\ H_{124}\ N_{22}\ O_{16}\ S_2$ (MH^+) 1858.9. Amino acid analysis: Glx 2.12 (2); Pro 1.97 (2); Gly 1.00 (1); Leu 1.99 (2); Nle 0.97 (1); His 0.90 (1); Arg 0.98 (1). Bpa can not be determined.
10. In the synthesis of Bpa^{13} analog (compound 2) %S calculated 3.07; found %S 3.16.
11. Similar differences in the retention times, UV adsorbance plots and 76 Da difference in mass was found for all other Bpa-containing analogs of α-factor.
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13. Green, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis;* John Wiley and Sons, Inc.: New York, 1991; pp. 198-207.
14. Haroutounian, S.A., Synthesis 1995, No 1, 39-40.
15. Cleavage mixtures: 1) TFA/H_2O (97.5%/2.5% v/v), or 2) TFA/H_2O/EDT (95/2.5/2.5%). For consistency in our experiments we maintained the proportion of cleavage mixture-to-peptide resin as 12.5 μl/mg. Cleavage was carried out at room temperature for 1.5 hours. Products were purified by HPLC. 1) Ac-Ala-Bpa-Leu; ES-MS (m/z) Found: 496.5; Calculated for $C_{27}\ H_{33}\ N_3\ O_6$ (MH^+) 496.2; 2) 1,3-Dithiolane derivative of Ac-Ala-Bpa-Leu; ES-MS (m/z) Found: 572.4; Calculated for $C_{29}\ H_{37}\ N_3\ O_5\ S_2$ (MH^+) 572.2.
16. Ac-Ala-Bpa-Leu was treated at 5°C using conditions proportional to conditions of ref. 6. Typically for of the kinetic experiments @ 5°C. Ac-Ala-Bpa-Leu-OH (6 μmol) was dissolved in 150 μl of TFA/H_2O/dithiol mixture (95/2.5/2.5 % v/v). Monitoring of the reaction was performed using the HP-1090 HPLC system with autosampler. Conditions of the analysis: thermostabilized (5°C sample) autosampler compartment, μ-Bondapack C_{18} 3.9 x 300 mm column, isocratic separation with 50% acetonitrile/water (0.025% TFA) mixture, flow rate 1.5 ml/min. Monitoring with diodarray detector.
17. Percent thioketal formation equals mmoles of C divided by mmoles of (A+C) multiplied by 100.
18. Li, C.H., Yamashiro, D., *J.Am.Chem.Soc.,* **1970,** *92,* 7604 - 7608.
19. March, J. *Advanced Organic Chemistry;* John Wiley and Sons, Inc.: New York, 1992; p.894. This product is not stable.

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