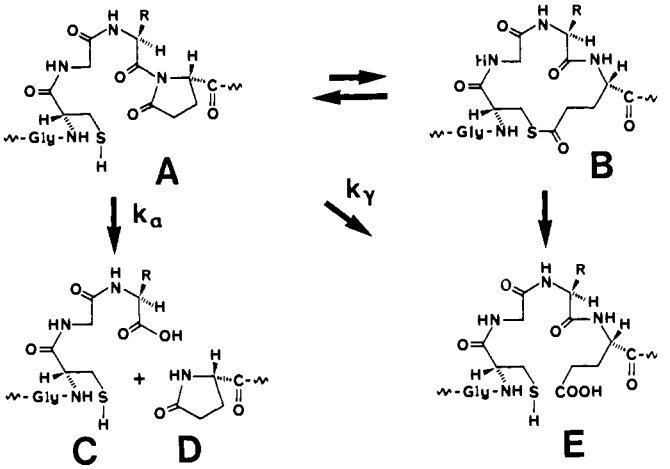
to override our chemical expectations. In the present case, interactions between HLE and the peptide moiety of MeOSuc-Ala-Ala-Pro-Val are sufficiently strong in the acylation transition state that acylation of HLE by the *p*-nitroanilide of this peptide proceeds faster than hydrolysis of the acyl-enzyme, MeOSuc-Ala-Ala-Pro-Val-HLE. Furthermore, while such interactions between HLE and MeOSuc-Ala-Ala-Pro-Val may not be strong enough to cause rate-limiting deacylation for the primary amide of this peptide, when leaving group subsite structural requirements are fulfilled sufficient stabilization of the transition state for amide acylation does occur, again resulting in rate-limiting deacylation.

Registry No. MeOSuc-Ala-Ala-Pro-Val-pNa, 70967-90-7; MeOSuc-Ala-Ala-Pro-Val-ONP, 88425-48-3; Phe-NH_2, 5241-58-7; elastase, 9004-06-2.

Scheme I



Synthesis and Regioselective Hydrolysis of Peptides Containing an Internal Residue of Pyroglutamic Acid

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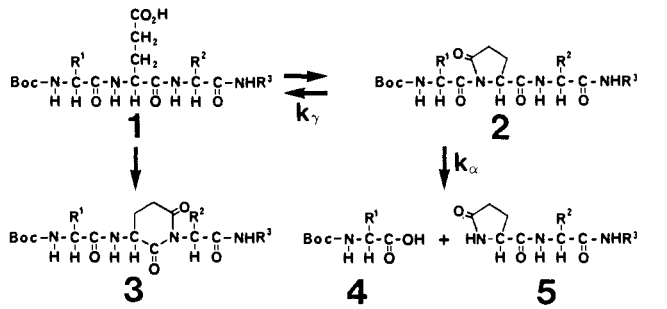
Efficient, mild, and rather general procedures are described for conversion of internal glutamic acid (Glu) residues into internal pyroglutamic acid (Glp; 5-oxoproline) residues and regioselective hydrolysis of the latter mainly with peptide chain fragmentation. The metastable binding sites of complement components C3[1] and C4[2] and protease inhibitor α_2-macroglobulin[3] evidently exist as macrocyclic thiolactone B, R = $CH_2CH_2CO_2H$ (Scheme I). Under denaturing conditions they undergo spontaneous hydrolysis[4] with fragmentation. Since product D contains a preformed N-terminal Glp residue, a plausible intermediate is lactam A containing an *internal* Glp residue. Synthetic hexapeptide models of lactam A having R = CH_3 or $CH_2CH_2CO_2H$ undergo chain fragmentation (k_α) to C and D in preference to ring opening (k_γ) to E.[5-7]

Several methods for preparing small peptides containing internal Glp residues (activation of Glu with thionyl chloride[8] or ethyl chloroformate;[9] permanganate oxidation[10] of proline) are reported to give low yields and byproducts. Conversion of an internal residue of Glu benzyl ester[11] or Glu sugar ester[6] to Glp in liquid HF, however, proceeds in moderate to good yield. Seven Boc-tripeptide amides (**1a-g**, R = Bzl, Scheme II) were synthesized. Typically, mixed anhydride coupling of Boc-Glu(OBzl) with Val-NH_2 followed by acidolysis of the Boc group gave Glu(OBzl)-Val-NH_2 (92% yield), which was coupled with Boc-Ala mixed anhydride to afford Boc-Ala-Glu(OBzl)-Val-NH_2 in 94% yield. Catalytic transfer hydrogenolysis[12] of the latter furnished Boc-Ala-Glu-Val-NH_2 in 95% yield. Half esters **1c,d**, R = Bzl, were made by the scheme[5] for Boc-Glu(OBzl)-Glu-Asn-NH_2.

Scheme II



a, $R^1 = CH_3$; $R^2 = CH_2CONH_2$
b, $R^1 = CH_2CH_2SCH_3$; $R^2 = CH_2CONH_2$
c, $R^1 = CH_2CH_2CO_2R$; $R^2 = CH(CH_3)_2$
d, $R^1 = CH_2CH_2CO_2R$; $R^2 = CH_2CONH_2$
e, $R^1 = H$; $R^2 = CH(CH_3)_2$
f, $R^1 = CH_3$; $R^2 = CH(CH_3)_2$
g, $R^1 = CH_2CH(CH_3)_2$; $R^2 = CH(CH_3)_2$
h, $R^1 = CH(CH_3)CH_2CH_3$; $R^2 = H$

Cyclization. Tripeptides **1** (Scheme II) were activated with *N,N'*-carbonyldiimidazole (CDI) and allowed to cyclize to give tripeptides **2**.[13] Typically, solid CDI (0.20 g, 1.2 mmol) was added to Boc-Gly-Glu-Val-NH_2 (**1e**, 0.40 g, 1.0 mmol) in DMF (2.0 mL) at −20 °C. The solution was stirred at −20 °C for 0.5 h and at 20 °C for 1.0 h and concentrated under reduced pressure. Addition of ether precipitated crude **2e**, which was purified by reprecipitation from DMF/ether. Half esters **1c,d**, R = Bzl, were treated with CDI to form ester lactams **2c,d**, R = Bzl, and deprotected by hydrogenolysis[12] to obtain acid lactams **2c,d**, R = H, which were purified by chromatography on octadecyl-silica. Yields of **2** were about 80% (Table I). Each of the peptides **1** and **2** showed the expected $(M + Na)^+$ ion in the ^{252}Cf fission

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Table I. Synthesis and Regioselective Hydrolysis of Seven Boc-Tripeptide Amides Containing an Internal Residue of Pyroglutamic Acid

	peptide 2, R = H		k' value[a]			hydrolysis[b]			yield, %	
code	structure	yield, %	1	2	5	k_α	k_γ	k_α/k_γ	5	1
a	Boc-Ala-Glp-Asn-$NHCH_3$	83	0.45	1.06	0.15	100	5.4	18.3	94.9	5.1
b	Boc-Met-Glp-Asn-$NHCH_3$	84	1.60	3.01	0.15	59	4.5	13.1	92.9	7.1
c	Boc-Glu-Glp-Val-NH_2	81	1.36	2.29	0.24	27	2.0	13.2	93.0	7.0
d	Boc-Glu-Glp-Asn-$NHCH_3$	78	0.38	0.93	0.15	36	3.5	10.2	91.1	8.9
e	Boc-Gly-Glp-Val-NH_2	79	0.99	1.72	0.24	51	8.4	6.0	85.8	14.2
f	Boc-Ala-Glp-Val-NH_2	86	1.44	2.52	0.24	56	9.5	5.9	85.5	14.5
g	Boc-Leu-Glp-Val-NH_2	82	2.54	4.73	0.18	14	3.5	4.1	80.4	19.6

[a] Relative retention time on octadecyl-silica, where $k' = (t_{peptide}/t_{solvent}) - 1$ and t is the retention time in 6% CH_3CN containing 0.05% CF_3CO_2H (except 12% CH_3CN for g). [b] Apparent first-order rate constants (10^{-6} s^{-1}) for hydrolysis of 2 in 150 mM NaCl/10 mM Na phosphate at 37 °C and pH 8.3 (except pH 8.0 for 2c and 2d).

fragment-induced mass spectrum[14] and gave a characteristic 300-MHz proton magnetic resonance spectrum. Formation of lactams **2a–g** was accompanied by <1% of imides **3a–g** as measured chromatographically. Reaction of Boc-Ile-Glu-Gly-NH_2 (**1h**) with CDI, however, provided only 2% of the five-membered lactam **2h** and 95% of the six-membered imide **3h**. The extent of this alternate mode of cyclization is evidently determined by the relative bulk of side chains R^1 and R^2 of the flanking residues.

Hydrolysis. Apparent first-order rate constants k_γ for ring opening at the Glp γ carbonyl and k_α for chain fragmentation at the α carbonyl of the preceding residue were measured for peptides **2**, R = H, in phosphate-buffered saline at pH 8.0 or 8.3 and 37 °C. Solutions were analyzed over 5–6 h by reverse-phase liquid chromatography monitored at 220 nm (Table I). Corrected peak areas of lactams **2** and their hydrolysis products **1** and **5**[15] were used to calculate their mole fractions at various times and rate constants k_α and k_γ. The latter varied with the bulk of side chains R^1 and R^2. Replacement of Ala-1 by the larger residue Glu decreased k_α by 2- or 3-fold and k_γ by 2- or 5-fold, and replacement by Leu decreased k_α by 7-fold. Only modest rate changes were seen on substitution of Ala-1 by the smaller residue Gly or of Asn-3 by the β-branched residue Val. The regioselectivity ratio k_α/k_γ varied from 4.1 to 18.3 and did not generally correlated with the magnitude of k_α (compare **2c** and **2f**). In all seven cases, hydrolysis proceeded with 80–95% regioselectivity through attack at the α carbonyl group.[17]

Model Studies. The hydrolytic rate constants for these model tripeptides are similar to those we have observed[7] at pH 7.3 and 37 °C for synthetic hexapeptide lactams of type A, R = CH_2C-H_2CO_2H ($k_\alpha = 32 \times 10^{-6}$ s^{-1}, $k_\gamma = 3.7 \times 10^{-6}$ s^{-1}) or CH_3 ($k_\alpha = 65 \times 10^{-6}$ s^{-1}, $k_\gamma = 7.0 \times 10^{-6}$ s^{-1}). In both cases, hydrolysis proceeded with 90% regioselectivity, which is typical of an internal Glp residue (Table I).

Many peptide hormones, such as thyroliberin[18] (TRH), luliberin[19] (LH-RH), serum thymic factor[20] (FTS), and neurotensin[21] bear an N-terminal Glp residue. The present model studies suggest that some of these residues might also arise by regioselective hydrolysis with chain fragmentation of a precursor polypeptide containing an internal Glp residue.

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(15) Peptides **5a** and **5g** obtained by fragmentation of **2a** and **2g**, respectively, were identical with authentic[16] Glp-Asn-NH-CH_3 and Glp-Val-NH_2, respectively, by 300-MHz proton NMR spectroscopy and mass spectrometry.

(16) Z-Glp-Val-NH_2, prepared in 89% yield by mixed anhydride coupling of Z-Glp with Val-NH_2, was deprotected by catalytic transfer hydrogenolysis to furnish Glp-Val-NH_2 in 89% yield. Similarly prepared were Z-Glp-Asn-NH-CH_3 (77% yield) and Glp-Asn-NH-CH_3 (96% yield).

(17) Hydrolysis of a benzoyl dipeptide or tripeptide amide containing an internal Glp residue gave the fragment having an N-terminal Glp residue in 36–50% isolated yield.[8,9]

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"S_2": Generation and Synthetic Application

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Although reference to singlet oxygen ($^1O_2$) first appeared in the literature in 1924,[2,3a] it is primarily during the past two decades that most of its chemistry has been delineated.[3] The recognition that this form of molecular oxygen might play a central role in many of the important oxygen-related biological processes[3,4] has served to catalyze a current widespread interest in its chemical and biochemical reactivity. For several years now, in anticipation that singlet sulfur ($^1S_2$) might emulate singlet oxygen chemistry, we, among others, have been actively pursuing possible synthetic avenues for its preparation. We herein describe a procedure for the facile generation of "S_2"[5] and its application via the Diels–Alder reaction to the synthesis of cyclic disulfides.

Among the many procedures available for the generation of singlet oxygen ($^1O_2$),[3a,6] one of the most attractive is by means of the controlled, thermally induced decomposition of a phosphine or phosphite ozone adduct. These are conveniently prepared from

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