The progressive loss of potency in all assay systems as the hydroxyproline residue is moved toward the carboxyl end of the chain is impressive. This effect is consistent with the suggestion[12] that the initial combination of BK with its receptors is at the carboxyl end of the peptide chain, followed by the "zippering up" of the remainder of the peptide with the receptor. Any molecular modification which interferes with this initial peptide-receptor interaction should be extraordinarily effective in lowering the biological activity of hormone analogs. The biological activities of the 7-alanine, 7-D-proline, and 7-hydroxyproline analogs are quite similar. The very low activity of [Hyp^7]-BK may mean either that there is no space on the receptor to accommodate the increased bulk of the hydroxyl group or that the hydroxyl group interacts strongly with areas of the tissue adjacent to the receptor to prevent normal peptide-receptor combination. In any case, the change in biological activity upon replacement or proline by hydroxyproline is apparently not due to gross alteration in the conformation of the peptide. Among these analogs, biological activity is not correlated with the height of the 220-nm CD maximum.

Experimental Section

Synthesis of peptides was by the solid-phase method[5] using an automatic instrument similar to the one previously described.[13] Polystyrene–2% divinylbenzene (200–400 mesh beads) was substituted with Boc-Arg(NO_2) to the extent of 0.31 mmol/g. *tert*-Butyloxycarbonylamino acids were used throughout, including Boc-Ser(Bzl), and were purchased from Schwarz BioResearch, except *tert*-butyloxycarbonyl-4-hydroxyproline, which was purchased from Fox Chemical Co. Boc groups were removed during synthesis by 30-min treatment with 4 *M* HCl in dioxane containing 1 mg/ml of 2-mercaptoethanol, following a prewash of the same reagent. Peptide hydrochlorides were neutralized by 10-min treatment with 10% Et_3N in $CHCl_3$ (v/v), following a prewash. DCC mediated coupling reactions were done in $CHCl_3$, using 2.5 equiv of *tert*-butyloxycarbonylamino acids and DCC per equivalent of peptide-resin. Boc-Arg(NO_2) was coupled in 50% DMF–$CHCl_3$ because of its solubility. Peptides were cleaved from the resin by 30-min treatment at 0° with anhydrous HF containing 10% $PhOCH_3$.

Peptides were purified by countercurrent distribution for 100 transfers in 1-BuOH–trifluoroacetic acid (1%); observed partition coefficients are given in Table I. Purified peptides were homogeneous by paper electrophoresis[5] at pH 5 (E_{Lys} = 0.64) and pH 2.8 (E_{Lys} = 0.70) and had the same mobility as BK. In tlc on cellulose in 1-BuOH–AcOH–H_2O (4:1:5) the pure peptide trifluoroacetates showed two spots, R_f 0.44 and 0.69; standard BK trifluoroacetate had R_f 0.49 and 0.72. The slower spot is peptide acetate, while the faster spot is undissociated peptide trifluoroacetate. Amino acid ratios were determined on hydrolysates (6 *N* HCl, 110°, 22 hr) with a Beckman 120 amino acid analyzer and are given in Table I.

CD spectra were determined on a Cary 60 spectropolarimeter with a CD attachment. Peptides were dissolved in 0.03 *M* K_2HPO_4 adjusted to pH 7.2 with HCl. The instrument was standardized with an aqueous solution of *d*-10-camphorsulfonic acid. Spectra were checked for conformity to Beer's law.

Bioassays on isolated rat uterus,[14] isolated guinea pig ileum,[14] and rat blood pressure[15] were done as previously described.

For determination of the effect of the peptides on vascular permeability, albino guinea pigs weighing 300–400 g were etherized lightly and injected intracardially with Evans Blue dye (10 mg/kg as 0.25% solution in 0.9% NaCl). The back of the animal was clipped closely, but not shaved or depilated. Graded doses of the peptides were injected intradermally into the back area in 0.05 ml of phosphate buffered saline, pH 7.25 (8.5 g of NaCl, 0.40 g of Na_2HPO_4, and 0.10 g of $NaH_2PO_4 \cdot H_2O$ per liter). New disposable No. 27 needles were used only once to minimize skin trauma. Saline blanks and BK controls were included in every animal. Twenty minutes after injection of the peptides, the major and minor diameter of each spot was measured with calipers, moistening the skin if necessary for better visualization. The major and minor diameters of each spot were multiplied together to give the "area product," and from this value was subtracted the area product of the blank, which was usually 2–3 mm^2. The square root of the remainder was the "corrected diameter," and a plot of this value against the logarithm of the dose gave the dose–response curve, which was linear over a log dose range of at least 3. Results from animals not showing linear dose–response curves were not used. The slopes of the curves from the Hyp-BK analogs were parallel to those for BK. To test analogs for inhibition, a mixture of the analog and BK was injected, due to the difficulty of making successive intradermal injections into exactly the same spot. Analogs were tested for inhibition at concentrations below the threshold of BK-like response, covering a log dose range of at least 4.

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Analogs of the Abortifacient Aminoglutethimide

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Aminoglutethimide (1) has recently[1,2] been reported to induce abortions in pregnant rats by interfering with their cholesterol to Δ^5-pregnenolone conversion. The compound is nonsteroidal and nonestrogenic; thus it represents a new type of antifertility agent. Aminoglutethimide was found to clear from all tissues quickly with a half-life of 70 min. At suboptimal doses no gross abnormalities were noted in the surviving rat fetuses. An an abortifacient, aminoglutethimide might be used in humans only after a missed menses. This would mean it would be given less frequently than current steroidal combination drugs. The spread between efficacy (100 mg/kg) and toxicity (200 mg/kg), however, was not very great.[2] These reports prompted us to undertake an investigation to see if a more effective and less toxic analog could be made. A search of the literature revealed that most of the analogs reported were of

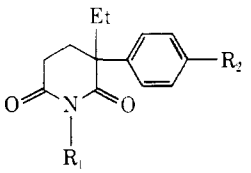
Table I. Preparation of Aminoglutethimide (1) Analogs

Compd no.	Reaction: Reagent	Solvent	Temp, °C	Time, hr	Yield, %	Mp, °C[a]	Recrystn solvent	Molecular formula[b]
			A. Aminoglutethimide Derivatives					
3	PhNCO	PhH	80	2.5	100	117–120	PhH–EtOH	$C_{20}H_{21}N_3O_3 \cdot 0.25C_6H_6$
4	PhCOCl–pyridine	Et_2O	0	0.5	58	233–234	EtOH	$C_{20}H_{20}N_2O_3$
5	Ac_2O	MeOH	25	1	67	187–188[c]	EtOH	$C_{15}H_{18}N_2O_2$
6	MeNCS	PhH	80	2	50	190–192	DMF–H_2O	$C_{15}H_{19}N_3O_2S$
7	EtNCO	PhH	80	2	100	195–196	EtOH	$C_{16}H_{21}N_3O_3$[d]
8	$(CF_3CO)_2O$–Na_2CO_3	Et_2O	0	1	85	245–247	EtOH	$C_{15}H_{15}F_3N_2O_3$
			B. Schiff Bases of Aminoglutethimide					
9	PhCHO	EtOH	78	0.5	58	161–163	$CHCl_3$–cyclohexane	$C_{20}H_{20}N_2O_2$
10	*p*-$AcNHC_6H_4CHO$	EtOH	78	0.5	56	231–233	EtOH–$CHCl_3$	$C_{22}H_{23}N_3O_3$
11	$Me_2NCH(OEt)_2$	EtOH	78	2	59	161–162	EtOH	$C_{16}H_{21}N_3O_2$
			C. N-Substituted 2-Ethyl-2-(*p*-nitrophenyl)glutamides (12)					
13	MeI	DMF	0	15	82	114–115	EtOH	$C_{13}H_{16}N_2O_4$
14	EtI	DMF	0	48	62	75–76	EtOH	$C_{15}H_{16}N_2O_4$
15	*m*-PrI	DMF	0	48	69	69–70	EtOH	$C_{16}H_{20}N_2O_4$
16	$PhCH_2Br$	DMF	0	48	60	101–102	EtOH	$C_{20}H_{20}N_2O_4$
			D. N-Substituted 2-(*p*-Aminophenyl)-2-ethylglutarimide (1)[e]					
17	H_2	HOAc			83	106–107	EtOH	$C_{14}H_{18}N_2O_2$
18	H_2	HOAc			96	Oil		$C_{15}H_{20}N_2O_2$[f]
19	H_2	HOAc			97	Oil		$C_{16}H_{22}N_2O_2$[f]
20	H_2	HOAc			100	Oil		$C_{20}H_{22}N_2O_2$[f]

[a] All melting points are uncorrected. [b] All compounds were analyzed for C, H, N, and if present F and S. The results obtained for those elements were within ±0.4% of the theoretical value. [c] Hoffmann[3] gave mp 183–185°. [d] C: calcd, 63.35; found, 62.90. [e] Prepared from the appropriate nitro precursor by hydrogenation at 2–3 atm using 5% Pd on charcoal. [f] The structures were confirmed by nmr as a good analysis could not be obtained.

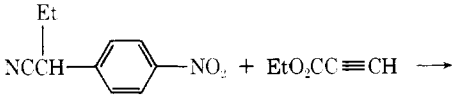
glutethimide (2) and only two amino analogs, the *p*-amino (1) and the *o*-amino derivative, had been reported.[3]

A series of derivatives 3–8 was prepared using standard reagents under the conditions described in Table IA. Likewise, a series of Schiff bases 9–11 was prepared from 1 and benzaldehyde, *p*-acetamidobenzaldehyde, and *N,N*-dimethylformamide dimethyl acetal, respectively (Table IB). Alkylation of 2-ethyl-2-(*p*-nitrophenyl)glutarimide[3] (12) with alkyl halides using sodium hydride in dimethylformamide gave 13–16 (Table IC) which were hydrogenated to 17–20, respectively (Table ID).



	R_1	R_2		R_1	R_2
1	H	NH_2	11	H	$N{=}CHN(CH_3)_2$
2	H	H	12	H	NO_2
3	H	NHCONHPh	13	CH_3	NO_2
4	H	NHCOPh	14	C_2H_5	NO_2
5	H	$NHCOCH_3$	15	*n*-C_3H_7	NO_2
6	H	$NHCSNHCH_3$	16	CH_2Ph	NO_2
7	H	$NHCONHCH_2CH_3$	17	CH_3	NH_2
8	H	$NHCOCF_3$	18	C_2H_5	NH_2
9	H	N=CHPh	19	*n*-C_3H_7	NH_2
10	H	$N{=}CHPhNHCOCH_3$	20	CH_2Ph	NH_2

An unsaturated ring system was prepared by condensing 2-(*p*-nitrophenyl)butyronitrile (21) with ethyl propiolate to give 22 as a mixture of *cis*- and *trans*-ethyl and -methyl esters. The methyl esters came from transesterification with the methanol present in the catalyst, *N*-benzyltrimethylammonium hydroxide (40% in methanol). Direct acid cyclization of 22 did not work here; thus, mild saponification was used to make 23 as a mixture of cis and trans acids, which was cyclized without purification to 24 using polyphosphoric acid. Since catalytic reduction might have destroyed the double bond, chemical reduction using sodium sulfide was used to make 25. Attempts to add nucleophiles in a Michael fashion to 25 failed, as did attempts to add chlorine or bromine.



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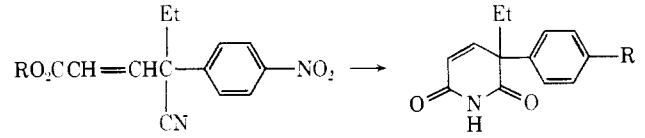
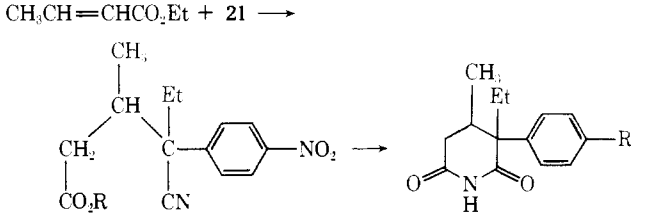


22, R = Et, Me
23. R = H
24. R = NO_2
25. R = NH_2

Substitution in the 3 position, 29, was achieved by the following scheme.



26, R = Et, Me
27. R = H
28, R = NO_2
29, R = NH_2

Ethyl crotonate was condensed with 2-(*p*-nitrophenyl)-butyronitrile (21) using benzyltrimethylammonium hydroxide (40% in methanol) to give 26, a mixture of ethyl and methyl esters as before. Saponification of 26 gave 27, which was cyclized using polyphosphoric acid to 28. Hydrogenation reduced the nitro group to give 29.

To test these analogs for antifertility activity, female Royal Hart rats, 210–230 g, were cohabited with proven breeders for 5 days. Starting on the fifth day, the females were dosed with compound for 10 days. The next day the rats were killed and examined for implantations, fetuses, and *corpora lutea*. Doses of 0, 50, 100, and 200 mg/kg/day (ip or subcutaneously) were used. In this test aminoglutethimide was active but all our analogs were found inactive. Thus it is concluded that the activity of 1 is very specific, as all of the changes made caused the activity to be lost.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, the results obtained for those elements were within ±0.4% of the theoretical values. The structures of all compounds were assigned on the basis of compatible ir and nmr spectra.

Ethyl (and Methyl) 4-Cyano-4-(*p*-nitrophenyl)-2-hexenoate (22). A solution of 28.5 g (0.15 mol) of 2-(*p*-nitrophenyl)butyronitrile[3,4] and 14.7 g (0.15 mol) of ethyl propiolate in 100 ml of dioxane was maintained at 60–80° under N_2 while 15 ml of benzyltrimethylammonium hydroxide (40% in methanol) was added dropwise. Upon completion of the addition, the dark solution was refluxed for 19 hr. The reaction mixture was distilled, accompanied by a great deal of decomposition, to give 20.9 g (48%) of a yellow oil, bp 188–192° (0.15 mm). While the elemental analysis was acceptable, partition chromatography showed at least three major products in roughly equal amounts. Identification by nmr showed them to be the cis and trans isomers of **22** and the *trans*-methyl ester analog of **22** (resulting from transesterification). Scaling up this reaction gave lower yields. *Anal.* ($C_{15}H_{16}N_2O_4$) C, H, N.

4-Ethyl-4-(*p*-nitrophenyl)glutaconimide (24). Because of the losses on distilling the starting material, crude ester **22** was used. A mixture of 141 g (0.50 mol in theory) of crude, black *cis*- and *trans*-ethyl and -methyl 4-cyano-4-(*p*-nitrophenyl)-2-hexenoate, 150 ml of EtOH, 40 g (1.0 mol) of sodium hydroxide in 200 ml of H_2O, and 100 ml of THF was stirred for 0.5 hr. Then 200 ml of H_2O was added and stirring continued for another 2.5 hr. Upon acidification with aqueous HCl, the mixture was extracted twice with Et_2O (the volume of Et_2O was noted as the interface was not visible). Filtration of the combined organic extracts through diatomaceous earth removed some solid matter. Next the product was extracted into aqueous $KHCO_3$ and the aqueous phase washed with EtOAc followed by acidification. After extraction with Et_2O, the solution of product was washed with brine, dried (Na_2SO_4), and evaporated to dryness. To the residual black oily acid was added 1000 g of polyphosphoric acid. The mixture was stirred and heated at 130–140° for 3 hr and then poured onto ice. Next, it was neutralized with aqueous concentrated KOH. A brown precipitate was collected and washed with H_2O, aqueous $KHCO_3$, and then H_2O. Air drying overnight gave 60.7 g (47%) of a dark brown solid. Four recrystallizations from ethanol and work-up of the mother liquors gave 19.4 g (15% from **21–24**) of yellow crystals, mp 175–178.5°. The nmr was consistent with the structure. *Anal.* ($C_{13}H_{12}N_2O_4$) C, H, N.

4-(*p*-Aminophenyl)-4-ethylglutaconimide (25). Heat was applied to a solution of 10.40 g (40 mmol) of 4-ethyl-4-(*p*-nitrophenyl)glutaconimide (**24**) in 50 ml (50 mmol) of 1 *N* NaOH, 20 ml of H_2O, and 14.8 g of wet (*ca.* 55 mmol) $Na_2S \cdot 9H_2O$ on a steam bath for 30 min. The resulting black solution was adjusted to pH 6 with aqueous HCl and cooled overnight. A brown precipitate was collected and leached twice with warm, dilute, aqueous HCl. Upon adjusting the pH of the extract to 6 with $NaHCO_3$ a tan precipitate appeared which was collected and dried to give 6.71 g (73%) of solid. Two recrystallizations from ethanol purified the product. Some unreacted starting material was recovered from the first brown precipitate which was recycled. A total of 4.09 g (44%) of colorless crystals, mp 200–202°, was obtained, whose nmr was consistent with the structure. *Anal.* ($C_{13}H_{14}N_2O_2$) C, H, N.

Ethyl (and Methyl) 3-Methyl-4-cyano-4-(*p*-nitrophenyl)hexanoate (26). This was prepared similarly to **22** except the benzyltrimethylammonium hydroxide (40% in methanol) used was twice taken up in *t*-BuOH and concentrated *in vacuo* before use in this reaction. A 50% yield of product was obtained as a yellow oil, bp 188–191° (0.15 mm). The vpc showed two major and one minor peaks, while the nmr showed 83% ethyl ester and 17% methyl ester, both being 50–50 mixtures of epimers. *Anal.* ($C_{16}H_{20}N_2O_4$) C, H, N.

2-Ethyl-2-(*p*-nitrophenyl)-3-methylglutaramide (28). This reaction was carried out on **26** like the preparation of **24** to give an overall 27% yield of solid product (EtOH), mp 143–150°. The structure of the mixture of diastereomers was confirmed by nmr. *Anal.* ($C_{14}H_{16}N_2O_4$) C, H, N.

2-(*p*-Aminophenyl)-2-ethyl-3-methylglutarimide (29). This reduction was carried out similarly to **17–20.** The product was a yellow oil whose structure and solvation were confirmed by nmr. *Anal.* ($C_{14}H_{18}N_2O_2 \cdot 0.25EtOH$) C, H, N.

Acknowledgment. We thank Dr. John A. Coppola and staff for carrying out the biological tests. Microanalyses were performed by Mr. L. M. Brancone and staff. Spectra were determined by Mr. W. W. Fulmor and staff. Interpretation of nmr spectra was carried out by Mr. G. Morton. All melting points and boiling points are uncorrected. Partition chromatography was carried out by Dr. C. A. Streuli's group.

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Studies on β-Lactams. 35.[1] Antibacterial Activity of Monocyclic β-Lactams

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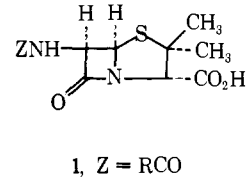
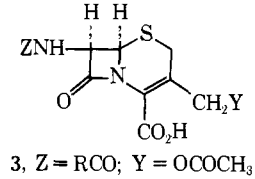
In recent years a large number of penicillins (**1**) and cephalosporins (**3**) have been prepared from 6-APA (**2**) and 7-ACA (**4**) in an effort to modify the antibacterial activity of these bicyclic β-lactams. From the literature on structure–activity correlation in the penicillin–cephalosporin field, it appears to be a generally held view that the following features are essential for therapeutically effective antibiotic activity:[2] an α-amido-β-lactam, cis stereochemistry of the fused β-lactam, and a free carboxy group in the non-β-lactam heterocycle part.





1, Z = RCO
2, Z = H

3, Z = RCO; Y = $OCOCH_3$
4, Z = H; Y = $OCOCH_3$

The various monocyclic β-lactams obtained by the scission of the thiazolidine ring in penicillins and subsequent transformations for obtaining cephalosporin derivatives appear to be without antibacterial activity.[3] We wish to report here the first examples of synthetic monocyclic β-lactams that have shown antibiotic activity.

In the course of a project on the total synthesis of diverse analogs of penicillins and cephalosporins, we have prepared variously substituted monocyclic β-lactams. The "acid chloride–imine" reaction[4] was used for the synthesis of the 1,3,4-trisubstituted 2-azetidinones described herein.