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## Synthesis and Biological Evaluation of $\omega$ -Homologues of Prostaglandin E<sub>1</sub>

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The synthesis and biological activities of some compounds with novel modifications of the  $\omega$  side chain of prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) are described. The preparation of ( $\pm$ )- $\omega$ -Me-PGE<sub>1</sub> (3), ( $\pm$ )- $\omega$ -Et-PGE<sub>1</sub> (4), ( $\pm$ )- $\omega$ -Pr-PGE<sub>1</sub> (5), and ( $\pm$ )- $\omega$ -Bu-PGE<sub>1</sub> (6) is outlined. The compounds were evaluated for in vitro smooth muscle stimulating activity on isolated gerbil colon preparations, for hypotensive action in anesthetized rats, and for gastric antisecretory effects in histamine-stimulated Heidenhain pouch dogs. Structural changes in the  $\omega$  position of the noncarboxyl side chain of PGE<sub>1</sub> influenced the biological potency of the resulting compound when compared to the reference standard PGE<sub>1</sub> (2). The homologues demonstrated interesting separation of biological activities; for example, 4 showed potent hypotensive activity (84% of PGE<sub>1</sub>) but, unlike PGE<sub>1</sub>, it showed very low smooth muscle stimulating activity. Compound 3 possessed the same order of potency as 2 in the gastric antisecretory assay.

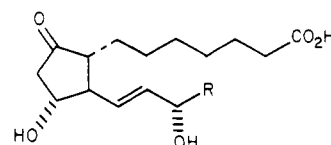
Simple chemical modification of a hormonal substance, or a drug, profoundly modifies its biological activity. A number of currently known drugs are either methyl homologues or desmethyl analogues of known compounds. Examples are methyl-Dopa, epinephrine, norgestrel, and most oral contraceptives chemically defined as 19-nor-progestins. Such changes in biological activity may sometimes be predicted based on the inhibition of metabolic degradation.

Labhsetwar<sup>1</sup> showed that a C-22 derivative of PGF<sub>2 $\alpha$</sub>  was 20 times more potent than the parent PGF<sub>2 $\alpha$</sub>  in terminating early pregnancy in hamsters when administered orally. Beerthuis et al.<sup>2</sup> prepared (Scheme I)  $\omega$ -nor-PGE<sub>1</sub>, PGE<sub>1</sub>, and  $\omega$ -homo-PGE<sub>1</sub> by biosynthesis from the corresponding unsaturated fatty acids and determined some aspects of structure and activity relationships (SAR) for these compounds on platelet aggregation, isolated guinea pig ileum, and hypotensive effects in the anesthetized rat.

To elucidate more salient SAR in the  $\omega$ -homo series, we have synthesized ( $\pm$ )- $\omega$ -Me-PGE<sub>1</sub> (3), ( $\pm$ )- $\omega$ -Et-PGE<sub>1</sub> (4), ( $\pm$ )- $\omega$ -Pr-PGE<sub>1</sub> (5), and ( $\pm$ )- $\omega$ -Bu-PGE<sub>1</sub> (6) and tested<sup>3</sup> their activities on the gerbil colon, blood pressure of the anesthetized rat, and gastric secretion in the dog. The chemical structures are shown in Scheme I.

**Chemistry.** The synthesis of ( $\pm$ )-3, -4, -5, and -6 is outlined in Scheme II. The starting aldehyde tetrahydropyranyl ether (7) prepared by a previously published procedure<sup>4</sup> was condensed with an appropriate phosphorane (8) to give 15-dehydro- $\omega$ -homo-PGE<sub>1</sub> tetrahydropyranyl ethers (9). Reduction of 9 with thexyl tetrahydrolimonyl borohydride followed by acid hydrolysis

Scheme I. Chemical Structures of  $\omega$ -Homologues of PGE<sub>1</sub>



- 1, R =  $n$ -C<sub>5</sub>H<sub>11</sub>
- 2, R =  $n$ -C<sub>5</sub>H<sub>11</sub>
- 3, R =  $n$ -C<sub>6</sub>H<sub>13</sub>
- 4, R =  $n$ -C<sub>7</sub>H<sub>15</sub>
- 5, R =  $n$ -C<sub>8</sub>H<sub>17</sub>
- 6, R =  $n$ -C<sub>9</sub>H<sub>19</sub>

Table I. Properties of Phosphoranes (C<sub>6</sub>H<sub>5</sub>)<sub>3</sub>P=CHCOR

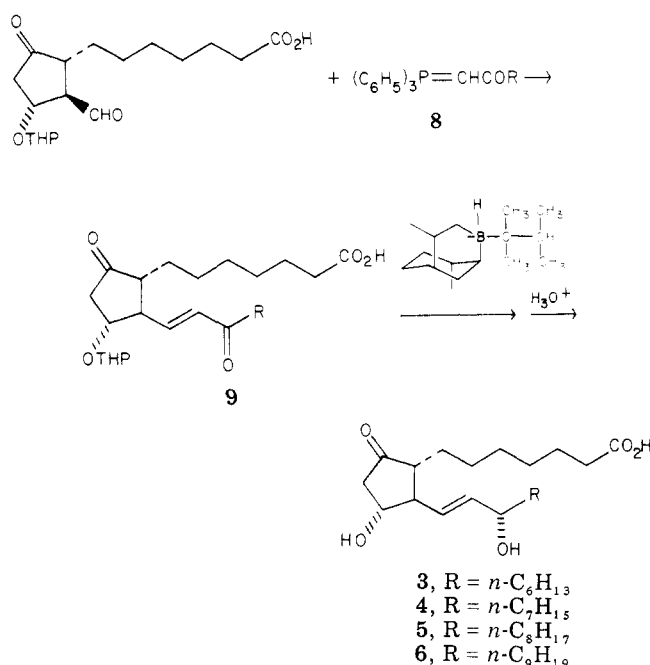
R	Mp, °C (recrystn solvent)	Formula	Anal- yses
$n$ -C <sub>6</sub> H <sub>13</sub>	86 (benzene-Skelly B)	C <sub>26</sub> H <sub>29</sub> OP	C, H
$n$ -C <sub>7</sub> H <sub>15</sub> <sup>a</sup>	83 (Skelly B)	C <sub>27</sub> H <sub>31</sub> OP	C, H
$n$ -C <sub>8</sub> H <sub>17</sub> <sup>b</sup>	202 (EtOAc)	C <sub>28</sub> H <sub>34</sub> OPCl	C, H
$n$ -C <sub>9</sub> H <sub>19</sub>	78 (Skelly B)	C <sub>29</sub> H <sub>35</sub> OP	C, H

<sup>a</sup> See ref 4. <sup>b</sup> Characterized as hydrochloride.

produced the ( $\pm$ )- $\omega$ -homologues of PGE<sub>1</sub> (3-6). The phosphoranes (8) required for the synthesis were prepared by the imidazolid procedure.<sup>5</sup> Their physical and chemical properties are summarized in Table I.

Compounds 3-6 were pure crystalline substances and each exhibited a single spot on a thin-layer plate [silica gel, benzene-ethyl acetate-acetic acid (25:25:1), sprayed with ethanolic phosphomolybdic acid]. That they have "natural" configurations was confirmed by comparison of

Scheme II



THP = tetrahydropyranyl

**Table II.** Relative Potencies of the  $\omega$ -Homologues of Prostaglandin  $E_1$  on Inducing Contractions on Isolated Gerbil Colon Preparations

Compd	Potency rel <sup>a</sup> to $PGE_1$	95% confidence limits
2 ( $PGE_1$ )	1.0	
3 [(±)- $\omega$ -Me- $PGE_1$ ]	0.54	0.45-0.67
4 [(±)- $\omega$ -Et- $PGE_1$ ]	0.064	0.056-0.076
5 [(±)- $\omega$ -Pr- $PGE_1$ ]	0.005	0.003-0.006
6 [(±)- $\omega$ -Bu- $PGE_1$ ]	0.006	0.004-0.009

<sup>a</sup> Activities were determined using the maximum stimulation produced by the test compounds. Relative potencies were determined by four-point parallel line bioassays.

their 100-MHz  $^1\text{H}$  NMR spectra in deuteriomethanol with that of natural  $PGE_1$  (2) and its stereoisomers. The pattern of a fine multiplet at  $\delta$  5.69 (H-13,14) for 3-6 was indistinguishable from that of 2.

## Results and Discussion

The synthesis of  $\omega$ -homologues of  $PGE_1$  yields racemic compounds. It has been well established that the racemic prostaglandins possess half the biological potency of the naturally occurring enantiomers.<sup>6-8</sup> Hence, the absolute biological potency of the present prostaglandins should be multiplied by two when compared with natural  $PGE_1$  or the  $\omega$ -analogues synthesized by the Unilever group.<sup>2</sup>

The  $\omega$ -homologation of the  $PGE_1$  molecule, in a stepwise manner, influenced the potency of the resulting prostaglandins. As is evident in Table II, racemic  $\omega$ -Me- $PGE_1$  has approximately half the activity of the reference standard  $PGE_1$  in stimulating the isolated gerbil colon. The activity decreased considerably with the incorporation of additional methylene groups. The  $\omega$ -Et- $PGE_1$  showed only about 6% of the activity of  $PGE_1$  and the  $\omega$ -propyl and  $\omega$ -butyl analogues were essentially inactive. This finding suggests that the  $\omega$  side chain must not be increased more than one carbon to maintain appreciable smooth muscle stimulating activity in the gerbil colon.

The hypotensive activities of the  $\omega$ -homologues did not parallel the *in vitro* isolated gerbil colon findings. As is evident in Table III, the  $\omega$ -Me- and  $\omega$ -Et- $PGE_1$  had nearly

**Table III.** Relative Hypotensive Potencies of  $\omega$ -Homologues of Prostaglandin  $E_1$  in the Anesthetized Rat

Compd	Potency rel <sup>a</sup> to $PGE_1$	95% confidence limits
2 ( $PGE_1$ )	1.0	
3 [(±)- $\omega$ -Me- $PGE_1$ ]	0.68	0.44-0.90
4 [(±)- $\omega$ -Et- $PGE_1$ ]	0.84	0.69-1.03
5 [(±)- $\omega$ -Pr- $PGE_1$ ]	0.42	0.32-0.54
6 [(±)- $\omega$ -Bu- $PGE_1$ ]	<0.12 <sup>b</sup>	

<sup>a</sup> The values were determined using the maximum hypotensive responses after *iv* bolus injections of  $PGE_1$  and the test compounds. Relative potencies were determined by four-point parallel line bioassays. <sup>b</sup> Very weak and inconsistent biological response. The relative potency value was obtained by comparison of individual responses.

**Table IV.** Gastric Antisecretory Actions of  $\omega$ -Homologues of  $PGE_1$  in the Dog

Compd	$ED_{50}^a \pm SE$ , $\mu\text{g/kg iv}$	Potency $\pm SE$ rel to $PGE_1$
2 ( $PGE_1$ )	$7.59 \pm 1.38$	1.00
3 [(±)- $\omega$ -Me- $PGE_1$ ]	$5.8 \pm 3.01$	$1.13 \pm 0.5$
4 [(±)- $\omega$ -Et- $PGE_1$ ]	$\sim 30$	$\sim 4$
5 [(±)- $\omega$ -Pr- $PGE_1$ ]	<i>b</i>	<i>b</i>
6 [(±)- $\omega$ -Bu- $PGE_1$ ]	<i>c</i>	<i>c</i>

<sup>a</sup> Represents the dose needed to reduce total acid output by 50%. <sup>b</sup> The compound was totally inactive at the highest tested dose of 30  $\mu\text{g/kg iv}$  bolus in two dogs. An insufficient supply of compound precluded testing at higher doses. <sup>c</sup> Marginal and inconsistent antisecretory activity was noted at 100  $\mu\text{g/kg iv}$  bolus. Higher doses were not attempted.

equal hypotensive activity to  $PGE_1$ , while  $\omega$ -Pr- $PGE_1$  showed half the activity of  $PGE_1$ . In view of the racemic composition of these compounds, it is expected that the natural isomers of compounds 3 and 4 would be two times more potent than  $PGE_1$  as hypotensive agents. The  $\omega$ -Bu- $PGE_1$  was found to be considerably less active than  $PGE_1$ .

The gastric antisecretory activities of  $PGE_1$  homologues appeared to be strongly dependent on the structure of the compound (Table IV).  $\omega$ -Me- $PGE_1$  had a nearly identical  $ED_{50}$  value to  $PGE_1$ . On the other hand, increasing the side chain of  $PGE_1$  by more than one methylene group decreased the potency. The  $\omega$ -Et- $PGE_1$  has one-fourth the potency of  $\omega$ -Me- $PGE_1$ . The  $\omega$ -propyl was inactive at the highest tested *iv* bolus dose of 30  $\mu\text{g/kg}$ . The  $\omega$ -Bu- $PGE_1$  showed marginal activity at the 100  $\mu\text{g/kg iv}$  bolus dose.

These studies with  $PGE_1$  homologues demonstrated interesting separation of biological activities. For example, (±)- $\omega$ -Et- $PGE_1$  appeared to have peak hypotensive activity in the anesthetized rat assay. This racemate had 84% the activity of  $PGE_1$ . The resolved material would be expected to have about 170% of the activity of  $PGE_1$ . Despite such peaks in activity for this ethyl homologue in both the hypotensive assay and the platelet aggregation test,<sup>3</sup> this substance had only low smooth muscle activity as measured by the gerbil colon assay.

The synthetic incorporation of appropriate alkyl groups in the C-20 position of  $PGE_1$  might be expected to interfere with possible  $\omega$  oxidation occurring at that position.<sup>9</sup> The  $\omega$ -homologues appear to be important in developing more biologically selective prostaglandins.

## Experimental Section

**Chemistry.** (±)-9,15-Dioxo-11 $\alpha$ -tetrahydropyranyl-13-*trans*-prostenoic Acids (9). To a solution of 20 g of 7<sup>4</sup> in 50 ml of methylene chloride was added 300 ml of freshly prepared

Table V. Properties of  $\omega$ -Homoprostaglandin  $E_1$ 

Compd	Mp, °C (recrystn solvent)	Formula	Anal- yses
3	100.5–101 (EtOAc)	$C_{21}H_{36}O_5$	C, H
4	80.5 (EtOAc-Skelly B)	$C_{22}H_{38}O_5$	C, H
5	91 (EtOAc-Skelly B)	$C_{23}H_{40}O_5$	C, H
6	82 (ether)	$C_{24}H_{42}O_5$	C, H

chromous sulfate solution<sup>10</sup> at 5–10 °C under nitrogen. The mixture was vigorously stirred at 25 °C for 30 min. To the green reaction mixture was added, in sequence with vigorous stirring, 30 g of ammonium sulfate, 230 g of sucrose, 130 ml of 1 M citric acid, and 500 ml of ether. The ethereal extract (total 2 l) was washed with 100 ml of saturated ammonium chloride solution and then with a saturated sodium chloride solution and dried over sodium sulfate. Upon evaporation of ether, 21 g of oily residue was obtained, which was treated immediately with 45 g of an appropriate phosphorane (8)<sup>5</sup> in 250 ml of benzene for 6 days at 25 °C. Often a small amount of tetrahydrofuran had to be added to get a homogeneous benzene solution. The reaction mixture was washed with cold 2% citric acid and then with 1% salt solution and dried over sodium sulfate. This concentration gave 30–36 g of residue, which was chromatographed on 800 g of SilicAR CC-4 using benzene that contained increasing percentages of ethyl acetate. The desired material (9, 10–13 g) was found in 10–15% ethyl acetate eluates which was used for the borohydride reduction without further purification. The characteristic brown color which developed on a silica gel thin-layer plate upon spraying with phosphomolybdic acid was the best criterion to locate 9.

( $\pm$ )- $\omega$ -Homoprostaglandin  $E_1$  (3–6). Over a period of 35 min, a solution of 20 mmol of hexyl tetrahydrolimonyllithium borohydride<sup>11</sup> in 65 ml of tetrahydrofuran-*n*-pentane was added to a solution of 5.5 g of 9 ( $R = C_6H_{13}$  or  $C_7H_{15}$ ) or 5.8 g of 9 ( $R = C_8H_{17}$  or  $C_9H_{19}$ ) in 50 ml of tetrahydrofuran at –78 °C under a nitrogen stream. The reaction mixture was immediately diluted with 100 ml of ether and treated with 10% citric acid while it was still cold. The ethereal extract was washed with 1% sodium chloride solution, dried over sodium sulfate, concentrated, and chromatographed on 500 g of SilicAR CC-4. The elution was carried out with benzene containing ethyl acetate. The tetrahydropyranyl ether of 3, 4, 5, or 6 was found in 25–35% of the ethyl acetate–benzene fractions (1.1–1.3 g) and free<sup>12</sup> 3, 4, 5, or 6 was found in 75–100% of the ethyl acetate–benzene fractions (0.3–0.6 g, crystalline). The tetrahydropyranyl ethers were hydrolyzed with acetic acid–water–tetrahydrofuran (20:10:3) for 24 h at 25 °C. Pure 3, 4, 5, and 6 were obtained by recrystallization from a solvent listed in Table V.

**Biological Studies. Drugs.** All compounds were dissolved in an isotonic sodium phosphate buffer solution<sup>13</sup> (pH 7.4) and stored at –10 °C when not in use.

**1. Isolated Gerbil Colon Studies.** Adult male gerbils (*Meriones unguiculatus*) were sacrificed by cervical dislocation. Segments of the ascending colon were removed and mounted in 2-ml glass-jacketed tissue baths maintained at 32 °C. The baths were filled with a low calcium bathing solution similar to that described by Gaddum<sup>14</sup> for isolated rat colon. The composition, in grams per liter, was NaCl 9.0, KCl 0.42,  $CaCl_2$  0.03,  $NaHCO_3$  0.15, and dextrose 1.0. It was bubbled with oxygen. Longitudinal contractions of the tissue were detected with Narco isotonic transducers (Model MK II) and reproduced on a Narco physiograph.

Contractions were elicited at 4-min intervals by two different doses of a test compound and two different doses of PGE<sub>1</sub> standard. The order of these additions was determined by a Latin square design. The tissue was rinsed after each contraction. Four contractions were elicited in response to each dose of test compound and PGE<sub>1</sub>. The relative potency of each prostaglandin in relation to the reference standard PGE<sub>1</sub> was determined by the method of Finney.<sup>15</sup>

**2. Blood Pressure Studies.** The hypotensive effects of the compounds were bioassayed using a previously described technique.<sup>16</sup> Briefly, male Charles River CD rats weighing 200–300 g were anesthetized with urethane (1.5 g/kg, intraperitoneally). Blood pressure was recorded via a cannulated carotid artery using a Narco (Model P-1000 A) pressure transducer and a Narco

physiograph. The femoral vein was cannulated for intravenous injection of the prostaglandins. Following surgery, the animals received heparin (5 mg/kg iv), followed by the subcutaneous administration of atropine sulfate (5 mg/kg) and pentolinium tartrate (10 mg/kg). Body temperature was maintained at  $32 \pm 0.5$  °C using a temperature controller (Yellow Springs Instrument Co., Model 73) and a 100-W light bulb as a heat source.

After blood pressure was stabilized, the test and the standard (PGE<sub>1</sub>) compounds were administered as a single iv bolus in a Latin square design. The maximum decrease in mean blood pressure following each injection was calculated and potency estimates were made using a four-point parallel line bioassay. Four to eight replications of each dose of standard and test compounds were recorded for each assay. Potency and confidence limits were estimated using the Finney<sup>15</sup> method.

**3. Gastric Antisecretory Studies.**<sup>17,18</sup> Six adult female mongrel dogs with Heidenhain pouches and weighing between 15 and 18 kg were used in these experiments. The dogs had been trained to stand quietly in a Pavlov support and were conscious during all studies. The surgery was done about six months before these studies were started. The animals were not used more than once per week.

Experiments were initiated by fasting the dogs for 18 h. On the morning of an experiment the dogs were placed in Pavlov stands and infused intravenously (iv) with 0.15 M NaCl solution. Gastric pouch secretion was collected at 15-min intervals and measured for volume to the nearest 0.1 ml. After 15–30 min of basal secretion, the dogs were infused with histamine solution at the submaximal dose of 1.0 mg/h. This dose, which was approximately equivalent to 60  $\mu$ g/kg/h, was chosen from predetermined dose–response curves to represent 75% of maximal stimulation in this series of dogs. The volume of infusion was kept at approximately 13.0 ml/h. Approximately 1 h after the start of histamine infusion, a steady-state plateau of gastric secretion was obtained. At this time the prostaglandin was administered by a single intravenous bolus injection using a total volume not exceeding 3.0 ml per dog. These doses usually ranged from 1.0 to 100  $\mu$ g/kg and were logarithmically spaced.

Gastric samples were measured for total acidity by titration with 0.1 N sodium hydroxide solution to pH 7.0 (Radiometer, Copenhagen). ED<sub>50</sub> values and relative potencies of the compounds were calculated<sup>15,19</sup> from the degree of inhibition of total acid output. The ED<sub>50</sub> is defined as the dose which caused 50% inhibition of total acid output in this series of dogs.

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## Tyrosylated Analogues of Somatostatin

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The total synthesis of [Tyr<sup>1</sup>]-, [Tyr<sup>6</sup>]-, [Tyr<sup>7</sup>]-, [D-Tyr<sup>8</sup>]-, [Tyr<sup>11</sup>]-, and [Tyr<sup>1</sup>,D-Trp<sup>8</sup>]somatostatin is described. The biological potencies of these analogues in vitro (inhibition of spontaneous secretion of radioimmunoassayable growth hormone by cultured rat anterior pituitary cells) as compared with that of somatostatin (100) were found to be 116, 29, 108, 10, 65, and 400, respectively; in vivo, in the rat, these peptides were assayed for their ability to inhibit the spontaneous release of insulin and glucagon. No statistically significant discrepancies were observed in the potency values obtained in vitro and in vivo further indicating similarities of specificities and sensitivities of the pituitary and pancreatic receptors.

The observation that des-(Ala<sup>1</sup>,Gly<sup>2</sup>)somatostatin or even des-(Ala<sup>1</sup>,Gly<sup>2</sup>)-desamino-Cys<sup>3</sup>-somatostatin were very potent analogues of somatostatin<sup>1</sup> (65 and 60%, respectively) led us to synthesize several acylated analogues of Cys<sup>3</sup>-somatostatin in an effort to obtain molecules which (a) could exhibit higher affinity for the receptor, which (b) might be more resistant to degradation by exopeptidases or that (c) could, because of their more hydrophobic character, be dissolved in oil and administered as a depot so that a protracted effect could be obtained. The biological evaluation in vitro and in vivo of such analogues has been reported by Brazeau et al.<sup>2</sup> and reevaluated by Brown et al.<sup>3</sup> Our conclusion was that the 38-membered ring of somatostatin contains all the information necessary for recognition and binding by the pancreatic and pituitary receptors and thus the side chain could be manipulated to yield tailored molecules. We predicted, for example, that [Tyr<sup>1</sup>]somatostatin would have full biological potency. This analogue, as well as other tyrosylated analogues of somatostatin, can easily be iodinated with radioactive I<sub>2</sub> or be specifically coupled by the bis-diazotized benzidine procedure<sup>4</sup> as hapten to larger proteins in order to raise specific antibodies against somatostatin (Vale et al., in preparation).

In view of the multiple possible use of tyrosylated analogues and as part of our studies on structure-activity relationships, we systematically substituted tyrosine for the phenylalanine residues at positions 6, 7, and 11.

The observation that [D-Trp<sup>8</sup>]somatostatin was eight times more potent than somatostatin in all systems tested<sup>5</sup> led us to synthesize analogues having other D- and L-amino acids at that position. The low biological potency of [Gly<sup>8</sup>]-, [L-Ala<sup>8</sup>]-, and [D-Ala<sup>8</sup>]somatostatin (<1%)<sup>6-9</sup> (and Rivier et al., in preparation) led us to conclude that, in contradistinction to luteinizing hormone releasing factor (LRF) for which substitution at the 6 position by any D-amino acids<sup>10</sup> yielded analogues as potent if not many times more potent than LRF, the indole ring at the eighth position of the somatostatin molecule was an absolute requisite for high biological potency. The synthesis of [D-Tyr<sup>8</sup>]somatostatin was to confirm this hypothesis.

[Tyr<sup>1</sup>,D-Trp<sup>8</sup>]somatostatin, combining two modifications, the first yielding a compound as active as somatostatin and the other making it superactive, was predicted to be more potent than somatostatin and could be used

in binding studies and characterization of the somatostatin receptor (Lazarus et al., in preparation).

This paper describes the total synthesis, physical constants, and biological evaluation in vitro and in vivo of materials that, through their ability of being easily radiolabeled, could be used in immunological, biological, and biochemical studies.

**Synthesis, Purification, and Characterization.** The tyrosylated analogues of somatostatin reported in Table I were synthesized by the solid-phase method<sup>11</sup> previously described for somatostatin<sup>12</sup> and analogues.<sup>1</sup> Boc-Cys-(SpOMe-Bzl) was esterified to the chloromethylated resin by a modification of Monahan and Gilon's procedure.<sup>13</sup> The stepwise buildup of the peptide on the resin was done using a standard procedure previously reported<sup>12</sup> with the exception that all couplings but those of Boc-Asn-PNP and Boc-Trp, for which dimethylformamide was used, were done in CH<sub>2</sub>Cl<sub>2</sub>.  $\alpha$ -tert-Butyloxycarbonyllysine ( $\epsilon$ -2ClZ)<sup>14</sup> was coupled at the ninth position while, for reasons of economy,  $\alpha$ -tert-butyloxycarbonyllysine ( $\epsilon$ -Z) was used at the fourth position. For threonine and serine, *O*-Bzl protection was chosen. For tyrosine the 2,6-Cl<sub>2</sub>Bzl protecting group turned out to live up to its promises.<sup>15</sup> When present in the peptide, alanine and glycine were introduced as the Z-protected dipeptide.

After cleavage and cyclization (potassium ferricyanide<sup>16</sup>), the peptides were purified by gel filtration<sup>17</sup> and counter-current distribution.<sup>18</sup> *R<sub>f</sub>* values on TLC in five different solvent systems or after paper electrophoresis, optical rotation, yields, and partition coefficient in a 1-butanol-acetic acid-water two-phase system are reported in Table II. Amino acid analyses<sup>19</sup> of peptide hydrolysates are reported in Table III.

**Biological Activity.** In vitro, the peptides were assayed for their ability to inhibit the spontaneous secretion of radioimmunoassayable growth hormone by rat anterior pituitary cells in monolayered culture according to Vale et al.<sup>20</sup> In vivo the peptides were assayed for their ability to inhibit the spontaneous release of insulin and glucagon in the rat. Test samples were injected in the jugular vein and blood samples collected from the hepatic portal vein of animals under ether anesthesia.<sup>21</sup> Hormone levels (growth hormone, insulin, and glucagon) were measured by specific radioimmunoassays.<sup>6</sup> Potency values were calculated by multiple dose bioassays with somatostatin