Solid-Phase Synthesis and Biological Properties of $\psi[CH_2NH]$ Pseudopeptide Analogues of a Highly Potent Somatostatin Octapeptide[1]

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A series of $\psi[CH_2NH]$ pseudopeptide analogues of a potent somatostatin octapeptide analogue, H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2, were synthesized by using a newly developed solid-phase method for direct introduction of the CH_2NH peptide bond isostere. Analogues were obtained in normal yields via a combination of sodium cyanoborohydride mediated reductive alkylation of resin-bound peptide amine with a *tert*-butoxycarbonyl amino acid aldehyde for introduction of a CH_2NH bond and normal solid-phase peptide chemistry. A racemization test on a model pseudodipeptide indicated that no more than 6% racemization took place during the aldehyde preparation and alkylation steps. Analogues were examined for their ability to inhibit growth hormone release in vivo in sodium pentobarbital anesthetized rats and in vitro from cultured rat anterior pituitary cells. Analogues modified at the amide carbonyl of Cys^2 and Lys^5 showed the highest in vivo activity (20 and 8 times more potent than SRIF, respectively) and in vitro activity (0.17 and 0.67 times as active as SRIF). This compared to in vivo and in vitro potencies of 8000% and 100%, respectively, for the parent analogue. Modification of the other cyclic ring amide carbonyls of Tyr^3, D-Trp^4, and Val^6 or the N- or C-terminal amide carbonyl of D-Phe^1 or Cys^7 gave analogues with considerably lower in vitro or in vivo potencies. The results appear to offer support for a proposed type II β-turn solution conformation centered on the D-Trp-Lys portion of SRIF octapeptide analogues, resulting in possible hydrogen-bonding interactions between Tyr^3 and Val^6 and D-Phe^1 and Thr^8 residues in the parent peptide. These would then be disrupted by methylene replacement of the carbonyl groups with concomitant loss of biological activity as was observed.

Intensive structure–activity and computer-modelling studies of somatostatin (SRIF) have resulted in the development of several shorter analogues with high biological potency and remarkable stability against proteolytic enzymes. Among these, the most documented are a cyclic octapeptide (SMS-201-995), H-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-ol,[2] and several cyclic hexapeptides,[3-5] such as *cyclo*(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe). Recently, other highly active cyclic octapeptide analogues have been reported[6-9] with various side-chain modifications, including the more active analogues H-D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2 and H-D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2. These compounds offered interesting targets for a recent rapid and resin-based method for synthesizing peptide analogues containing the CH_2NH bond isostere developed by Sasaki and Coy.[10] This involves use of the standard solid-phase technique in combination with reductive alkylation of a resin-bound amino group with a *tert*-butoxycarbonyl amino acid aldehyde with sodium cyanoborohydride[11] in acidified dimethylformamide. In the present paper, we describe the synthesis of a series of $\psi[CH_2NH]$ pseudopeptide analogues of the above octapeptide in which each individual or, in some instances, dual peptide bonds have been replaced by the reduced version, together with the abilities of the new peptides to inhibit in vivo and in vitro GH release in standard SRIF assay systems.

Experimental Section

General Methods. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured in a 0.5-dm cell on a Rudolph Research Autopol III automatic polarimeter. TLC was carried out on silica gel plates (Merck, silica gel 60) with the following solvent systems: R_f^1, AcOEt–*n*-hexane (1:2); R_f^2, AcOEt–*n*-hexane (1:1); R_f^3, 1-butanol–acetic acid–H_2O (4:1:1); R_f^4, 1-butanol–acetic acid–H_2O–AcOEt (4:1:1:1); F_f^5, 1-butanol–acetic acid–pyridine–H_2O (15:3:10:12). Reversed-phase HPLC was performed on a Vydac C_{18} silica (5 μm) column (4.5 mm × 30 cm) by gradient elution with the following solvent system: A, 5% acetonitrile in 0.1 M triethylammonium phosphate at pH 3.0 and B, 20% A in acetonitrile. A linear gradient from 5% B to 50% B over 30 min at a flow rate of 1.5 mL/min was used and the eluate was monitored at 215 nm. Purity of the synthetic octapeptides was assessed by HPLC with an LKB 2220 recording integrator. Amino acid analysis was carried out on a LKB-4150 analyzer using standard borate buffer methodology after peptide acid hydrolysis in 4 M methanesulfonic acid containing 0.1% tryptamine at 110 °C for 32–45 h in an evacuated sealed tube unless otherwise noted. Cys was determined as $Cys(SO_3H)$ by alternate treatment of samples with performic acid at 0 °C for 4 h followed by acid hydrolysis with 6 N HCl at 110 °C for 48 h.

Boc-amino Acid Aldehydes. These were synthesized *via* the corresponding Boc-amino acid *N*-methoxy-*N*-methylhydroxamides by the reportedly racemization-free $LiAlH_4$ reduction method described by Fehrentz and Castro.[12] Boc-D-Phe-CHO: mp 71–75

(1) Amino acids, peptides, and their derivatives were of the L configuration unless otherwise stated. Other abbreviations: $\psi[CH_2NH]$ = peptide bond replaced by a CH_2NH bond, D-Nal = 3-(2-naphthyl)-D-alanine, Boc = *tert*-butoxycarbonyl, Z = benzyloxycarbonyl, BrZ = 2-bromobenzyloxycarbonyl, Bzl = benzyl, MeBzl = 4-methylbenzyl, DCC = dicyclohexylcarbodiimide, TFA = trifluoroacetic acid, SRIF = somatostatin-14, HPLC = high-performance liquid chromatography, TLC = thin-layer chromatography, DMF = dimethylformamide, DMAP = (dimethylamino)pyridine.

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°C; $[\alpha]^{20}_D$ +20.4 (c 1.8, MeOH); R_f^2 0.70. Anal. ($C_{14}H_{19}NO_3$) C, H, N. Boc-Cys(MeBzl)-CHO: mp 69–71 °C; $[\alpha]^{20}_D$ −57.4 (c 1.1, MeOH); R_f^1 0.45, R_f^2 0.73. Anal. ($C_{16}H_{23}NO_3S$) C, H, N. Boc-Tyr(BrZ)-CHO: mp 34–36 °C; $[\alpha]^{20}_D$ −21.8 (c 1, MeOH); R_f^1 0.49. Anal. ($C_{22}H_{24}NO_6Br$) C, H, N. Boc-D-Trp-CHO and Boc-Lys-(Z)-CHO: These compounds were obtained as oils, and although some remaining hydroxamate was detected on TLC, they were used for coupling without further purification. Boc-D-Trp-CHO: R_f^2 0.72, Boc-Lys(Z)-CHO: R_f^2 0.42. Boc-Val-CHO: oil; $[\alpha]^{20}_D$ −12.8 (c 1.2, MeOH) [ref 12, $[\alpha]^{20}_D$ −19 (c 1, MeOH)]; R_f^1 0.60.

Peptide Synthesis. Solid-phase syntheses were carried out on a 1% cross-linked benzhydrylamine resin hydrochloride (0.3 mequiv/g) using a manual apparatus. Boc-amino acids with the side-chain protecting groups benzyl for Thr, 2-bromobenzyloxycarbonyl for Tyr, benzyloxycarbonyl for Lys, and 4-methylbenzyl for Cys were used. Boc-amino acids (Bachem, Inc.) (2.5 equiv) were coupled by the DCC method for 1 h. Completion of the reaction was checked by the ninhydrin test of Kaiser et al.[13] and, if necessary, repeated by the symmetrical anhydride method of Hagenmaier and Frank.[14] N^{α}-Boc deblocking was achieved with 33% TFA in CH_2Cl_2 for 5 and 25 min. After incorporation of the D-Trp residue, the deblocking was done with a mixture of 33% TFA in CH_2Cl_2 containing 3% anisole and 0.2% dithiothreitol. Ten percent triethylamine in CH_2Cl_2 was used for the neutralization (1 and 1 min).

Introduction of the CH_2NH bond was carried out as follows: after removal of the Boc group, the peptide resin trifluoroacetate was washed with DMF (×3) and the Boc-amino acid aldehyde (2.5 equiv) in DMF containing 1% AcOH was added, followed by the portionwise addition of $NaBH_3CN$ (2.5 equiv) over 40–60 min. The resin was checked by the ninhydrin test, and if necessary, the coupling reaction was repeated with the half equiv amounts of alkylating reagents. According to our preliminary study,[10] the secondary amine function of the CH_2NH bond has poor reactivity toward the DCC-mediated acylation with various Boc-amino acids except for Boc-Gly-OH. Thus, the resulting secondary amine function was not protected during the subsequent carbodiimide couplings. Protected octapeptide resins were cleaved from the resin and deprotected by treatment with anhydrous HF containing 10% anisole at 0 °C for 60 min. After evaporation of HF under a N_2 stream, the crude peptide was triturated with ether, collected by filtration, and washed with ether and then cyclized with I_2 in 90% AcOH (1 L/mmol of peptide).[15]

Purification. The crude octapeptides were purified on a column (2.5 × 90 cm) of Sephadex G-25, which was eluted with 50% AcOH followed by preparative medium-pressure chromatography on a column (1.5 × 42 cm) of Vydac C_{18} silica gel (15–20 μm), which was eluted with a linear gradient of 10–30% acetonitrile in 0.1% TFA with an Eldex Chromatrol gradient controller. Analogues I, II, IV, V, and VII–X were further purified by rechromatography on the Vydac C_{18} column with more prolonged gradient conditions. Eluate was monitored at 280 nm, and the main fractions were collected and lyophilized from H_2O. Homogeneity of the peptides was assessed by TLC and reversed-phase HPLC. Yields and analytical data are listed in Table II.

Identification of Isosteric Dipeptide Units. For authentic samples, isosteric dipeptide units were prepared by a classical solution method employing the $NaBH_3CN$-mediated reductive alkylation reaction.[16-18] A typical procedure for the solution synthesis is as follows. To a solution of Boc-amino acid aldehyde (1 mmol) and amino acid trifluoroacetate or amino acid methyl ester hydrochloride in MeOH containing 1% acetic acid (10 mL) was added $NaBH_3CN$ (1 mmol) over 40 min at room temperature.

Table I. Potencies of Synthetic ψ[CH_2NH] Pseudopeptide SRIF Analogues on Inhibition of Release of GH in the Anesthetized Rat and from Rat Pituitary Cells

bond replaced	% potency (SRIF = 100)[a] in vivo (95% confidence limits)	in vitro
SMS-201-995	8000[b]	500[c]
none (IM-IV-28)[d]	7900[b]	775[c]
I 1–2	160 (62–411)	1.01
II 2–3	1960 (874–4295)	17.43
III 3–4	20	0.68
IV 4–5	2	0.03
V 5–6	434 (171–1101)	67.27
VI 6–7	2	0.02
VII 7–8	73 (26–207)	0.41
VIII 2–3,5–6	65	0.98
IX 2–3,5–6[e]	50	3.44
X 4–5,6–7	2	<0.001

[a] See Tables V and VI for GH responses. [b] Taken from Murphy et al.[6] [c] Taken from Heiman et al.[9] [d] Parent sequence =

D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2
1 2 3 4 5 6 7 8 9

[e] Contains D-Nal in position 1.

After further stirring for 5 h, the solution was evaporated to dryness and extracted with 10% Na_2CO_3. The extract was washed with AcOEt and then acidified with solid citric acid to pH 4–5 with cooling. In most cases, desired product crystallized during the neutralization. The precipitate was collected and washed well with H_2O and MeOH and dried in vacuo. For the syntheses of Boc-D-Phe[CH_2NH]Cys(MeBzl)-OH and Boc-Cys(MeBzl)-[CH_2NH]Tyr-OH, DMF instead of MeOH was used as the reaction solvent. When an amino acid methyl ester was used as an amine component, the crude peptide methyl ester was saponified with 1 N NaOH/MeOH in a usual manner and then worked up as described above. The isosteric dipeptide compounds were deprotected by treatment with HF containing 10% anisole and used as reference standards for amino acid analysis. The Cys-[CH_2NH]Tyr unit in analogues II and IX was determined as H-Cys(SO_3H)[CH_2NH]Tyr-OH after performic acid oxidation and subsequent hydrolysis of the samples as described before. Retention times of standard isosteric dipeptide units on the amino acid analyzer were as follows: H-Phe[CH_2NH]Cys-OH, 55.9 min (just before Trp); H-Cys[CH_2NH]Tyr-OH, 55.1 min; H-Cys(SO_3H)[CH_2NH]Tyr-OH, 39.8 min (Met); H-Tyr[CH_2NH]D-Trp-OH, 61.3 min (Lys); H-D-Trp[CH_2NH]Lys, 80.8 min; H-Lys[CH_2NH]Val-OH, 62.3 min (just before NH_3); H-Val-[CH_2NH]Cys-OH, 55.1 min; H-Cys[CH_2NH]Thr-OH, 54.8 min (His). Physicochemical data of the intermediary dipeptide compounds are listed in Table IV.

Racemization. The model pseudodipeptide system D(L)-Phe-[CH_2NH]Leu-NH_2 was chosen for checking the extent of racemization during the overall procedure. Phe was chosen in view of its sensitivity generally to racemization and its use at the N-terminus of the present series of analogues. The dipeptides were synthesized from freshly prepared Boc-Phe-aldehydes on methylbenzhydrylamine resin by the standard reductive procedure and were cleaved by hydrogen fluoride treatment as already described. The dipeptide amides were then examined by HPLC using the triethylammonium phosphate system (0–20%, 30 min). D-Phe-[CH_2NH]Leu-NH_2 eluted at 9.3 min and the L diastereomer at 8.4 min. D-Phe-[CH_2NH]Leu-NH_2 contained 5.5% of the L diastereomer by integration of the peak areas.

Biological Assays. Peptides were tested as previously described[18] in male Spraque–Dawley rats which were anesthetized with sodium pentobarbital. Peptides were also screened in a 4-day primary culture of male rat anterior pituitary cells, and the detailed method has been described elsewhere.[19,20] GH in plasma or media was determined by radioimmunoassay using reagents generously supplied by the NIADDK. All data are expressed as the mean ± SEM (number of animals) (Table V). Relative in

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Table II. Yields and Analytical Data of $\psi[CH_2NH]$ Pseudooctapeptides

analogue	yield,[a] %	$[\alpha]_D$, deg (c 1, 1% AcOH)	TLC R_f^3	TLC R_f^4	HPLC purity, %	HPLC t_R, min
I	12	−50.2	0.32	0.59	95.5	19.1
II	12	−21.6	0.35	0.60	96.7	12.1
III	18	−59.8	0.29	0.58	96.0	13.0
IV	23	−7.2	0.24	0.59	98.0	13.0
V	8	−33.6	0.24	0.58	96.7	12.0
VI	12	−58.8	0.29	0.62	97.3	12.0
VII	6	−60.4	0.34	0.63	95.2	13.3
VIII	6	−21.2	0.21	0.58	95.1	8.5
IX	10	−25.8	0.22	0.59	95.0	13.0
X	8	+19.4	0.17	0.57	97.5	14.0

[a] Based on the starting resin.

Table III. Amino Acid Ratios of $\psi[CH_2NH]$ Pseudooctapeptides[a]

peptides	D-Phe	Cys[b]	Tyr	D-Trp	Lys	Val	Thr	NH_3	isosteres[c]
I	nd[d]	0.88	0.91	0.64	1.01	0.89	0.97	0.86	1.11
II	1.00	1.01	nd	0.53	0.97	1.05	0.82	1.35	1.03
III	1.00	1.17	nd	nd	0.98	0.77	1.05	0.97	+[e]
IV	1.00	1.20	0.54	nd	nd	0.42	0.94	0.91	0.37
V	1.00	1.64	0.93	0.53	nd	nd	0.99	1.08	0.63
VI	1.00	0.84	0.99	0.70	0.82	nd	0.92	1.00	0.73
VII	1.00	0.74	0.90	0.81	1.04	0.37	nd	0.94	0.84
VIII	1.00	0.61	nd	0.31	nd	nd	1.06	1.24	0.30,[f] 0.62[g]
IX	1.00[h]	0.61	nd	0.43	nd	nd	1.06	1.27	0.94,[i] 0.65[g]
X	1.00	0.52	0.76	nd	nd	nd	0.92	1.20	0.30,[j] 0.61[k]

[a] See Experimental Section for the acid hydrolysis of peptides and amino acid analysis. [b] Determined as $Cys(SO_3H)$. [c] See Experimental Section for the retention times on amino acid analyzer. [d] Not detected. [e] Eluted with Lys as a broad peak and not calculated. [f] H-Cysψ-$[CH_2NH]$Tyr-OH. [g] H-Lys$\psi[CH_2NH]$Val-OH. [h] D-Nal. [i] H-$Cys(SO_3H)\psi[CH_2NH]$Tyr-OH. [j] H-D-Trp$\psi[CH_2NH]$Lys-OH. [k] H-Valψ-$[CH_2NH]$Cys-OH.

Table IV. Physicochemical Data of Intermediary Isosteric Dipeptides

compound	mp, °C	$[\alpha]_D$, deg (c 0.5; DMF)	R_f^3	formula	anal.
Boc-D-Phe$[CH_2NH]$Cys(MeBzl)	161–163	+6.8	0.68	($C_{25}H_{34}N_2O_4S$)	C, H, N
Boc-Cys(MeBzl)$[CH_2NH]$Tyr	214–217[a]	+18.3	0.70	($C_{25}H_{34}N_2O_5S$)	C, H, N
Boc-Tyr$[CH_2NH]$D-Trp[b]	239–242[a]	+6.2	0.67	($C_{25}H_{31}N_3O_5$)	C, H, N
Boc-D-Trp$[CH_2NH]$Lys(Z)	180–182[a]	+17.4	0.69	($C_{30}H_{40}N_4O_6$)	C, H, N
Boc-Lys(Z)$[CH_2NH]$Val	190–192	−2.4[c]	0.61	($C_{24}H_{39}N_3O_6$)	C, H, N
Boc-Val$[CH_2NH]$Cys(MeBzl)	135–138	−5.3	0.65	($C_{21}H_{34}N_2O_4S$)	C, H, N
Boc-Cys(MeBzl)$[CH_2NH]$Thr	199–200	−24.4	0.58	($C_{20}H_{32}N_2O_5S$)	C, H, N

[a] Decomposition. [b] In contrast, the reaction of Boc-Tyr(BrZ)-CHO with H-D-Trp-OH gave the pseudodipeptide in only 22% yield, indicating less stability of the BrZ protecting group against $NaBH_3CN$-mediated reductive alkylation. [c] (c 1, MeOH).

vivo potencies (Table I) were determined for peptides on a weight basis by four-point assay[21] or, for less important analogues, by approximation based on comparison to typical SRIF dose responses. In vitro potencies (Table I) were determined as a ratio of the concentration of analogues producing 50% inhibition of GH secretion (EC_{50}) as compared to the EC_{50} of the standard. EC_{50} values (Table VI) were determined by using the computer program ALLFIT.[22] Both potency determinations used the tetradecapeptide SRIF molecule as the reference standard.

Results and Discussion

Synthesis. During the synthesis of the pseudopeptides, a $\psi[CH_2NH]$ bond replacement in the C-terminal portion (V and VII) or double replacements (VIII–X) tended to result in slightly lower yields as compared to N-terminal or single replacement, respectively (Table II). A possible reason for this may reside in increased small quantities of byproducts such as branched peptides arising from the newly formed secondary amine function as discussed in a previous paper.[10] Protection of this functional group by an appropriate group may give better yields, although no moiety has yet been found that is suitable for solid-phase synthesis conditions. On the whole, however, it could be concluded that peptides of this size range are well within the limitations of the method. An added advantage to the solid-phase coupling of aldehydes lies in the unreactivity and subsequent removal by rinsing of alcohol and nonreduced contaminants that are usually difficult to completely remove without resorting to SiO_2 chromatography.

The presence of the isosteric dipeptide units was demonstrated by acid hydrolysis of the peptides followed by comparison of their distinctive elution times with authentic samples on the amino acid analyzer. Their quantitative analysis data are listed in Table III together with amino acid ratios of the peptides. The amino acid ratios were in good agreement with the theoretical values except for some isosteric dipeptide units containing the acid hydrolysis sensitive indole ring and those amino acids adjacent to the isosteres. It should be noted that in peptide sequences having a $\psi[CH_2NH]$ bond, the neighboring peptide bonds are resistant to acid hydrolysis presumably due to steric and/or ionic factors of the isostere. After completion of this work, some concern was expressed generally and later by the reviewers of this paper about potential racemization problems inherent in the procedures used. The most dangerous step could be considered to be the generation of the hydroxamides using DMAP-catalyzed DCC couplings. Also the stability of amino acid aldehydes is somewhat suspect. We therefore undertook the synthesis of model stereoisomeric ψ[D- and L-Phe-L-Leu]NH_2 units using identical conditions to those used in the ana-

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Table V. GH-Release-Inhibiting Activities of $\psi[CH_2NH]$ Pseudooctapeptide SRIF Analogues in the Anesthetized Rat

test substance	dose, μg/100 g BW	plasma GH, ng/mL (no. of rats)
saline		316 + 41 (9)
SRIF	0.2	220 + 67 (5)
SRIF	0.6	133 + 20 (5)
I	0.2	185 + 39 (6)
I	0.6	90 + 13 (6)
VII	0.2	248 + 50 (5)
VII	0.6	156 + 20 (5)
saline		250 + 32 (9)
SRIF	0.2	163 + 39 (5)
SRIF	0.6	78 + 18 (6)
II	0.01	146 + 26 (5)
II	0.03	98 + 14 (4)
V	0.03	174 + 30 (6)
V	0.09	120 + 23 (5)
IM-IV-28	0.003	151 + 30 (6)
IM-IV-28	0.009	91 + 11 (6)
saline		261 + 27 (7)
III	0.1	307 + 50 (5)
III	1	176 + 23 (6)
III	10	137 + 10 (6)
saline		327 + 72 (8)
IV	0.1	325 + 53 (6)
IV	1	348 + 34 (5)
IV	10	265 + 28 (6)
saline		337 + 103 (7)
VI	0.1	381 + 67 (7)
VI	1	266 + 45 (6)
VI	10	225 + 21 (5)
saline		294 + 42 (9)
VIII	0.01	258 + 41 (5)
VIII	0.1	250 + 63 (5)
VIII	1	124 + 14 (5)
IX	0.01	433 + 78 (5)
IX	0.1	291 + 53 (5)
IX	1	133 + 20 (5)
saline		358 + 48 (6)
X	0.1	509 + 48 (6)
X	1	393 + 61
X	10	325 + 44 (6)

Table VI. In Vitro GH-Release-Inhibiting Activities of $\psi[CH_2NH]$ Pseudooctapeptide SRIF Analogues

peptides	ED_{50} + SEM[a]
SRIF	$6.1 \times 10^{-11} + 1.5 \times 10^{-11}$
I	$6.1 \times 10^{-9} + 2.5 \times 10^{-9}$
II	$3.5 \times 10^{-10} + 1.9 \times 10^{-10}$
III	$9.1 \times 10^{-9} + 1.8 \times 10^{-9}$
IV	$1.9 \times 10^{-7} + 1.0 \times 10^{-7}$
V	$9.2 \times 10^{-11} + 1.0 \times 10^{-10}$
VI	$3.5 \times 10^{-7} + 9.5 \times 10^{-8}$
VII	$1.5 \times 10^{-8} + 1.2 \times 10^{-8}$
VIII	$6.3 \times 10^{-9} + 1.6 \times 10^{-9}$
IX	$1.8 \times 10^{-9} + 5.0 \times 10^{-10}$
X	$>1.0 \times 10^{-6}$

[a] The molar concentration of peptide producing 50% inhibition of GH secretion.

logue studies and resolved them by HPLC. No more than 6% of contamination by the opposite isomer was observed, indicating that a small but perhaps acceptable degree of racemization did indeed occur. It might be possible to decrease this at the hydroxamate synthesis stage by eliminating the use of DMAP since, in our experience, the coupling reaction appears to proceed satisfactorily without catalysis. The long-term stability of Boc-amino acid aldehydes is an unknown factor, and experiments are in progress to address this problem also.

Structure–Activity Relationships. All the analogues tested in this study showed lower inhibitory potencies on GH release both in vivo and in vitro than the parent octapeptide (Table I, V, and VI). However, two analogues containing Cys[2][CH_2NH]Tyr[3] (II) and Lys[5][CH_2NH]Val[6] (V) replacements still showed high in vivo potencies (2000% and 400% relative to SRIF, respectively). In vitro potencies (Table VI) were always much lower than in vivo potencies. Indeed, the parent peptide itself and SMS-201–995 (Table I) only exhibited 8 and 5 times the potency of SRIF in this system[9] despite being 80 times more potent in the rat. We have also recently shown[9] that the receptor affinities of this type of analogue are seldom higher than that of SRIF. On the other hand, the potent octapeptide analogues that have been examined exhibit much prolonged activity in the rat[8] and in humans in the case of SMS-201–995. The analogues are clearly extremely resistant to proteolytic enzyme cleavage and are very lipophilic so that it can be assumed that these properties are responsible for high potencies in the rat compared to those in enzyme-free cell culture and membrane-receptor systems.

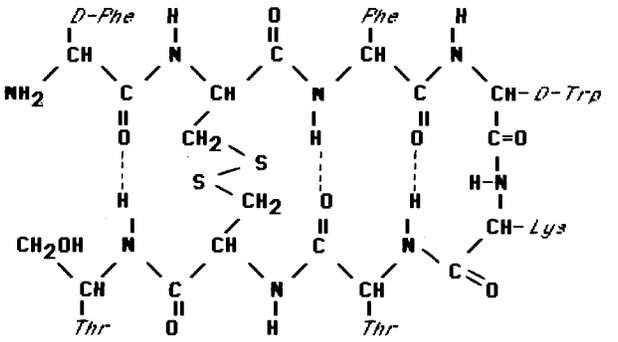

Figure 1. Proposed NMR solution conformation[24] for SRIF octapeptide SMS-201–995 encompassing a type II′ β-bend for positions 3–6 and hydrogen bond formation between D-Phe and Thr-ol (1–8) and Phe and Thr (3–7).

The other peptide bond replacements within the cyclic ring portion (III, IV, and VI) resulted in considerable loss of activity both in vivo and in vitro. Recently, it has been shown[24] in 500-MHz nuclear magnetic resonance studies in Me_2SO solution that SMS-201–995 probably has one predominant type II′ β-turn conformation involving the D-Trp-Lys sequence. This would favor hydrogen bond formation between CO and NH groups in positions 1–8 and 3–7. A qualitative representative of this model is shown in Figure 1. Our present biological results indeed demonstrate a seemingly greater importance of the cyclic ring amide carbonyls of positions 3, 4, and 6 and lesser contributions from the two carbonyls of Cys[2] and Lys[5], which seems consistent with this conformational model. The importance of the amide carbonyl of D-Trp[4] can be explained by assuming that it might contribute to a better proximity of the D-Trp[4] and Lys[5] side chains, which has been shown to correlate with higher biological activity in conformationally constrained SRIF analogues.[25,26] Combination of modifications of D-Trp[4] and Val[6] (X) or Cys[2] and Lys[5] carbonyls (VIII) gave analogues with no or only weak activities both in vivo and in vitro. Replacement of D-Phe in analogue VIII by D-Nal, while effective in the parent peptide,[8] gave an analogue (IX) that was only slightly improved in terms of in vitro activity while its in

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vivo activity was not altered. Isosteric modification of the N- or C-terminal peptide bond gave analogues (I and VII) with considerably decreased activities. For the D-Phe CO group, this is again consistent with hydrogen bond disruption. In any case, it seems likely that the lack of the two carbonyls of D-Phe[1] and Cys[7] might change the orientation of D-Phe[1] and Thr[8] residues, which may possibly stabilize the active conformation of the molecule as suggested for SMS-201–995 by Bauer et al.[2] and Wynants et al.[27]

It can be seen that the reduced peptide bond can be a useful probe for examining structure–activity-conformation relationships in biologically active peptides. The solid-phase method for introducing it into a particular sequence now makes it possible to make this type of analogue with no more effort than normal peptides. In addition, the NH group, while not highly reactive probably due to steric hindrance, can nevertheless be acylated under forcing conditions[10] or perhaps alkylated and thus offers a reactive center for producing further interesting conformational effects on peptide backbones.

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Registry No. I, 108104-67-2; II, 108104-68-3; III, 108104-69-4; IV, 108104-70-7; V, 108104-71-8; VI, 108104-72-9; VII, 108104-73-0; VIII, 108104-74-1; IX, 108104-75-2; X, 108104-76-3; GH, 9002-72-6; BOC-D-Phe-CHO, 77119-85-8; BOC-Cys(MeBzl)-CHO, 108104-77-4; BOC-Tyr(BrZ)-CHO, 108104-78-5; BOC-D-Trp-CHO, 108104-79-6; BOC-Lys(Z)-CHO, 82689-16-5; BOC-D-Phe-[CH_2NH]Cys(MeBzl)-OH, 108104-80-9; BOC-Cys(MeBzl)-[CH_2NH]Tyr-OH, 108104-81-0; BOC-Tyr[CH_2NH]D-Trp-OH, 108104-82-1; BOC-D-Trp[CH_2NH]Lys(Z)-OH, 108104-83-2; BOC-Lys(Z)[CH_2NH]Val-OH, 108104-84-3; BOC-Val[CH_2NH]-Cys(MeBzl)-OH, 108104-85-4; BOC-Cys(MeBzl)[CH_2NH]Thr-OH, 108104-86-5; H-Phe[CH_2NH]Cys-OH, 108104-87-6; H-Cys-[CH_2NH]Tyr-OH, 108104-88-7; H-Cys(SO_3H)[CH_2NH]Tyr-OH, 108104-89-8; H-Tyr[CH_2NH]D-Trp-OH, 108104-90-1; H-D-Trp-[CH_2NH]Lys-OH, 108104-91-2; H-Lys[CH_2NH]Val-OH, 108104-92-3; H-Val[CH_2NH]Cys-OH, 108104-93-4; H-Cys-[CH_2NH]Thr-OH, 108104-94-5; BOC-Val-CHO, 79069-51-5.

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Synthesis and Evaluation of Non-Catechol D-1 and D-2 Dopamine Receptor Agonists: Benzimidazol-2-one, Benzoxazol-2-one, and the Highly Potent Benzothiazol-2-one 7-Ethylamines

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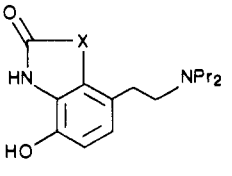
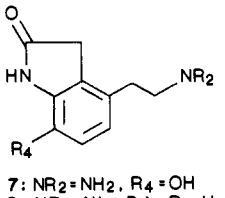
Our interest in identifying D-1 and D-2 dopamine receptor agonists that are not catechols led us to extend previous studies with oxindoles by investigating analogues of dopamine, *N,N*-dipropyldopamine, *m*-tyramine, *N,N*-dipropyl-*m*-tyramine, and epinine in which the *m*-hydroxyl is replaced by the NH portion of a thiazol-2-one, oxazol-2-one, or imidazol-2-one group fused to the 2,3-position. These compounds were evaluated for their affinity and agonist activity at D-1 and D-2 receptors by using in vitro assays. Replacement of the *m*-hydroxy in *N,N*-dipropyldopamine with the thiazol-2-one group resulted in a dramatic increase in D-2 receptor affinity and activity compared to that of *N,N*-dipropyldopamine itself or that of the corresponding oxindole, **1**. The resulting compound, 7-hydroxy-4-[2-(di-*n*-propylamino)ethyl]benzothiazol-2(3*H*)-one (**4**), is the most potent D-2 receptor agonist reported to date in the field-stimulated rabbit ear artery (ED_{50} = 0.028 nM). The benzoxazol-2-one (**6**), benzimidazol-2-one (**5**), and isatin (**51**) analogues showed D-2 receptor agonist potency similar to that of **1**. The des-7-hydroxy analogue of **4** (**21**) also has enhanced D-2 receptor activity compared to that of the corresponding oxindole, **8**. 7-Hydroxy-4-(2-aminoethyl)benzothiazol-2(3*H*)-one, **27**, a non-catechol, has enhanced D-1 and D-2 receptor activity in vitro compared to that of the corresponding oxindole, **7**. In vivo, **27** increased renal blood flow and decreased blood pressure in the dog. However, these effects were mediated primarily by D-2 receptor agonist activity. This may be a result of the D-1 partial agonist activity of **27** coupled with its potent D-2 receptor activity.

Previous publications from our laboratory have described the potent presynaptic D-2 (DA_2)[1] dopamine receptor agonist activity of 4-[2-(di-*n*-propylamino)ethyl]-7-hydroxy-2(3*H*)-indolone (**1**) and certain analogues.[4-6] Structure–activity relationships studies showed that **1** was the most potent D-2 agonist (EC_{50} = 1.8 nM for inhibition of vasoconstriction caused by electrical stimulation of the rabbit ear artery), while the racemic 3-methyl analogue **2** was 10 times less active and the 3,3-dimethyl analogue **3** was inactive. This suggested that an acidic hydrogen on the 3-position of the oxindole might be required for dop-



1: X = CH_2
2: X = $CHCH_3$
3: X = $C(CH_3)_2$
4: X = S
5: X = NH
6: X = O



7: NR_2 = NH_2, R_4 = OH
8: NR_2 = $N(n\text{-}Pr)_2$, R_4 = H

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