1,3-diketone containing a substituent on the methine carbon atom which can effectively stabilize a negative charge.[8] It is very reasonable to assume that the $Re(CO)_4$ group would stabilize a negative charge very effectively. Adding a drop of methanol-d_4 to the ^1H NMR solution of **1** in CS_2 leads to no change in the methyl resonance while the enol resonance completely disappears.

The acidity of the enol proton is qualitatively similar to that of acetylacetone. Although complex **1** is very soluble in hexane, it dissolves also in potassium carbonate–water, pyridine–ether, and sodium hydride–ether media with the appearance of the characteristic pale yellow color of the "metallo(acac)" anion. An ether solution of complex **1** does not evolve carbon dioxide from sodium bicarbonate, but spontaneously evolves hydrogen when placed over sodium hydride.

The keto tautomer of complex **1** is less favored, presumably, because this tautomer would be a seven-coordinate rhenium(III)–hydride complex, and the only complexes of this type which have been prepared have contained a η^5-cyclopentadienyl ligand. The ^1H NMR resonance of the hydride ligand in these complexes occurs at τ 12 or above.[10]

The investigation of the similarities in the organic reaction chemistry of complex **1** to the known reactions of 1,3-dicarbonyl molecules is being pursued.

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Supplementary Material Available: A listing of data, structure factor amplitudes, refinement procedures, least-squares planes, interatomic bond distances and angles, and positional and thermal parameters (18 pages). Ordering information is given on any current masthead page.

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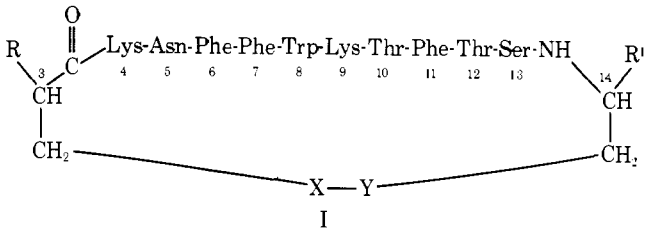
Nonreducible Cyclic Analogues of Somatostatin

Sir:

Since the isolation, characterization,[1] and synthesis[2] of the peptidal release inhibiting factor, somatostatin (Ia), the question concerning the biological activities of the cyclic and linear (reduced) forms has not been resolved, because the systems used to measure biological activities both in vitro and in vivo do not preclude interconversion of the cyclic (disulfide) and linear (dithiol) forms of somatostatin. The very low activity of [Ala^{3,14}]somatostatin[3,4] does not clarify the question of activity of dihydrosomatostatin since replacement of the two sulfurs by hydrogen may produce more consequences than the obvious ring opening. (See ref 4 for further discussion of these problems.) Convincing evidence that a cyclic form can possess intrinsic activity and that sulfhydryls are not required for activity would be obtained through the synthesis of a highly active cyclic analogue of somatostatin which cannot be cleaved by biological reduction. We have, therefore, undertaken the syntheses of somatostatin analogues in which one or both of the sulfur atoms have been replaced by methylene groups.[5] We report herein our initial findings with two analogues of somatostatin having both sulfur atoms replaced by methylene groups. A simplification of the synthetic objective was suggested by the observation that des(Ala^1,Gly^2)-desamino-[Cys^3]somatostatin (Ib) is highly active.[3,6] We therefore synthesized des(Ala^1,Gly^2)-desamino[Cys^3]dicarba^{3,14}-somatostatin (Ic) as a model of a nonreducible, cyclic somatostatin C-terminal analogue. In a further modification designed to test the requirement for the carboxyl group, we also synthesized des(Ala^1,Gly^2)-desamino[Cys^3]descarboxy-[Cys^{14}]dicarba^{3,14}-somatostatin (Id).



I

Ia, R = H-Ala-Gly-NH-; R' = CO_2H; X = Y = S (somatostatin)
b, R = H; R' = CO_2H; X = Y = S
c, R = H; R' = CO_2H; X = Y = CH_2
d, R = R' = H; X = Y = CH_2

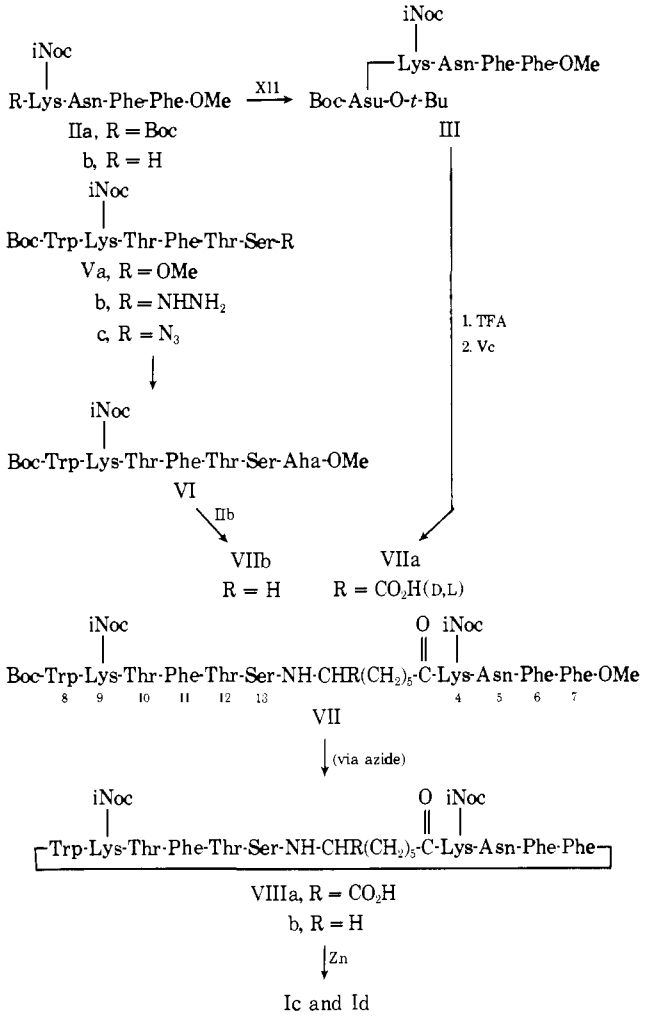
The synthetic routes to Ic and Id are outlined in Scheme I. It should be noted that the amino terminal sequence of the intermediates VII corresponds to positions 8–13 of somatostatin. Cyclization forms the amide bond required to convert these intermediates to a sequence equivalent to that of somatostatin.

The common intermediates, II and Va, were synthesized sequentially in solution as well as by solid phase methods. They were characterized by NMR and uv spectroscopy and amino acid analysis (Table I).

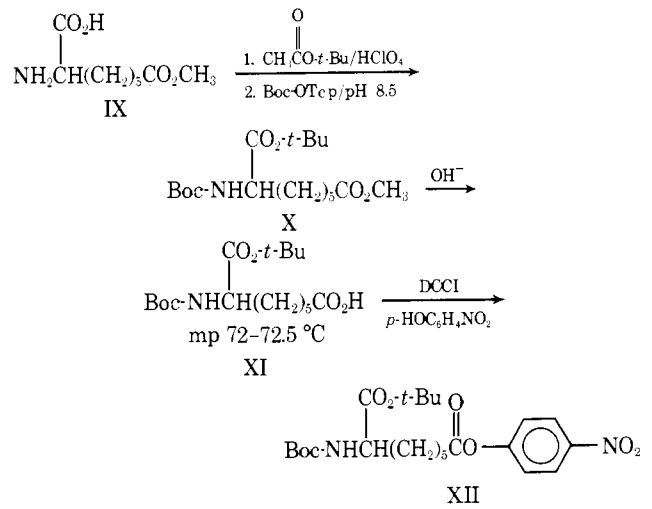
Toward the synthesis of Ic, the aminosuberic acid residue was incorporated by condensation of α-*tert*-butyl ω-*p*-nitrophenyl N^{α}-Boc-D,L-α-aminosuberate (XII) with the tetrapeptide IIb to give III. The selectively protected α-aminosuberic acid derivative XII was prepared as shown in Scheme II from ω-methyl-D,L-α-aminosuberate (IX).[9a,b] Both the Boc and *tert*-butyl ester protecting groups of III were removed by the action of trifluoroacetic acid. Coupling[10] of III with Vc gave VIIa, which was purified by gel filtration (Sephadex G-25, 50% acetic acid). VIIb was prepared by condensation of VI (via the azide) with IIb and purified in the same manner as VIIa. The ester VI was prepared by condensation of methyl ω-aminoheptanoate with Vc.

The undecapeptides VII were converted to hydrazides by the action of hydrazine in methanol, and the Boc protecting group was removed by HCl in ethyl acetate using mercaptoethanol as scavenger. The hydrazides were then converted to azides under acidic conditions.[10] Dilution of the resulting aminoazides to a concentration of 1 mg/ml with DMF at −20° and neutralization to pH 7.5 (as measured on moistened narrow range indicator papers) by the addition of diisopropylethylamine allowed cyclization to proceed. The crude VIII generated in this reaction was partially purified by precipita-

Scheme I[7]



Scheme II



tion from methanol with ethyl acetate. The iNoc protecting groups were removed from the amino groups of the lysines by the action of zinc dust in 50% aqueous acetic acid.[11] Purification of the resulting Ic and Id was accomplished by gel filtration through Sephadex G-25SF using 50% aqueous acetic acid as eluent.

Ic and Id were characterized by NMR (100 MHz) and uv spectroscopy and amino acid analysis (Table I). The points of elution of these two products from the final Sephadex column

Table I

	Amino acid analysis	$[\alpha]_{589}^{24}$ [a]	R_f [b] (solvent system)[8]
IIa	$Lys_{1.00}$, $Asp_{0.98}$, $Phe_{2.00}$	−24.1	0.76 (A), 0.79 (E), 0.93 (C)
Va	$Lys_{1.01}$, $Thr_{1.97}$, $Ser_{1.02}$, $Phe_{0.97}$, $Trp_{1.01}$	−29.0[d]	0.75 (B)
Ic	$Lys_{1.98}$, $Asp_{1.00}$, $Phe_{2.99}$, $Thr_{2.03}$, $Ser_{0.99}$, D,L-$Asu_{1.03}$,[f] $Trp_{0.74\ (1.0)}$[e]	−35.5	0.14 (C), 0.43, 0.49 (D),[f] 0.23 (E), 0.31 (F)
Id	$Lys_{1.93}$, $Asp_{1.02}$, $Thr_{2.03}$, $Ser_{1.05}$, $Phe_{2.96}$, $Trp_{0.61\ (0.97)}$[e]	−34.4	0.37 (C), 0.93 (D), 0.29 (E)
III	$Lys_{1.00}$, $Asp_{1.00}$, $Phe_{2.00}$, $Asu_{1.10}$		0.08 (B), 0.30 (C), 0.30 (E)

[a] c = 1 in 50% acetic acid (concentration is that of the peptide only, lacking salt, counterions, and solvates as determined by quantitative amino acid analysis permitting calculation of the molecular rotation). [b] Silica gel (Quantum Industries Type Q-1). [c] Solvent systems used: (A) CH_2Cl_2–*i*-PrOH; 95:5. (B) $CHCl_3$–MeOH–H_2O; 80:20:2. (C) EtOAc–Pyr–HOAc–H_2O; 10:5:1:3. (D) $CHCl_3$–MeOH–NH_4OH(concn)–H_2O; 50:40:6:4. (E) *n*-BuOH–HOAc–H_2O; 65:10:25. (F) *n*-PrOH–H_2O–NH_4OH(concd); 80:20:2. [d] c = 0.8 in 50% acetic acid. [e] Determined by uv. [f] Two components of about equal intensity were observed, consistent with a diastereomeric mixture resulting from the use of D,L-α-aminosuberic acid. The acid hydrolysate of the peptide was analyzed for the enantiomeric composition of α-aminosuberic acid by application of the method of Manning and Moore to D,L-α-aminosuberic acid [*J. Biol. Chem.*, **243**, 5591 (1968)]. The hydrolysate was allowed to react with the *N*-carboxyanhydride of L-glutamic acid. The ratio of diastereomeric dipeptides of α-aminosuberic acid was the same as that obtained from the starting D,L-α-aminosuberic acid within the accuracy of the method. Samples eluted from the plates showed D-Asu in the product having R_f 0.49 and L-Asu in the product having R_f 0.43.

Table II. Biological Activities: Inhibition of Gastric Secretion and Growth Hormone Release

	Gastric secretion[a]		
Compound	ED_{80}, μg/(kg min^{-1}) (95% C.L.)	Rel. pot. (95% C.L.)	Growth hormone[b] Rel. pot. (95% C.L.)
Somatostatin	0.06 (0.02, 0.10)	1.0	1.0
Ic	0.04 (0.03, 0.07)	1.35 (0.65, 2.54)	0.5 (0.08, 1.59)
Id	0.07 (0.02, 0.17)	0.89 (0.37, 1.91)	0.5 (0.31, 0.84)

[a] Studies in pentagastrin (2.5 μg/(kg h^{-1}) stimulated dogs. Compound introduced by iv infusion during steady state of gastric secretion. ED_{80} = dose required for 80% inhibition of total acid output. [b] Pentobarbital stimulated growth hormone release in the rat.

were consistent with monomeric structures. That no kinetic preference or subsequent separation of diastereomers had occurred during the synthesis of Ic was established by determination that the diastereomeric ratio of Ic was the same as the ratio of D to L in the starting D,L-α-aminosuberic acid (see Table I and footnote *f*).

Somatostatin has a broad spectrum of biological activities including inhibition of the release of growth hormone,[1] insulin, and glucagon with a net lowering of blood glucose,[12] and inhibition of gastrin-induced gastric secretion.[13,14] These parameters have been measured for somatostatin and the analogues Ic and Id.[15] The results of the studies of inhibition of pentagastrin induced gastric secretion (dog)[14] and inhibition of pentobarbital stimulated growth hormine release (rat)[16] are summarized in Table II. Intravenous infusion of Ic and Id into Rhesus monkeys results in a lowering of insulin, glucagon, and

blood glucose, as well as lowering growth hormone levels, the results being qualitatively similar to those observed with somatostatin.

In the gastric secretion studies it was noted that Id maintained the same inhibitory effect for about 30 min after the infusion had stopped, whereas the inhibitory effect of somatostatin and of Ic was lost shortly after the infusion had been discontinued. This prolonged duration of the action of Id may be a result of the elimination of those structural features which would lead to metabolism by aminopeptidases, carboxypeptidases, and reduction.

We conclude from the high biological activities of Ic and Id that the cyclic form of somatostatin can be active, that the disulfide bridge does not play a functional role, and that the presence of a terminal carboxyl group is not required for these activities.

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X-Ray Photoelectron Spectroscopic Studies of the Thermal Stability of Chlorophyll a Monohydrate

Sir:

The x-ray photoelectron spectrum (XPS) of porphyrins and related molecules has been of current interest due to the important structural details inherent in the N 1s, C 1s, and metal spectra.[1] An extension of XPS determinations to a characterization of chlorophyll (Chl) a-H_2O interactions appears to be desirable. Recently it has been inferred from optical and water titration data that the driest Chl a prepared from existing procedures (heating under vacuum up to 80 °C for a prolonged period) occurs as a monohydrate.[2] The intimate relationship between Chl a and H_2O lies at the heart of the photosynthesis problem.[3] It is believed that the primary molecular adduct P700[4] in photosystem I is a symmetrical dimeric aggregate of Chl a monohydrate.[3a,5]

The objective of the present work is twofold: (1) By XPS determination of the O 1s spectrum, we hope to make a direct experimental observation of the chlorophyll monohydrate. (2) Using XPS as a monitor, we hope to chart the course of the dehydration of the tightly bound[2] water as the temperature is incrementally raised above 80 °C, the temperature commonly employed as the upper limit in most drying procedures.[2,6]

The Chl a was extracted from spinach and purified in the usual manner.[2] Film preparation was accomplished using the sample preparation equipment available on the modified Hewlett-Packard 5950A ESCA spectrometer.[8] An atomically clean gold surface was transferred from the sample preparation chamber with a residual pressure 5×10^{-9} Torr into the attached N_2 atmosphere box. About 10^{15} Chl a molecules cm^{-2} were deposited on this gold surface by allowing 2 μl of a 3×10^{-4} M solution of Chl a in highly purified butyronitrite[9] × evaporate. The "dry" film was then inserted into the analyzer section of the ESCA instrument which has a residual pressure of 2×10^{-9} Torr. The film was thick enough so that no gold peaks were visible in the XPS spectrum. Slight charging effects (~1–2 eV) were partially compensated by using an electron gun[8] that floods the sample with low energy (<1 eV) electrons. Measurement of accurate peak positions is not possible using this approach, but the spectral distributions can be obtained with high accuracy. The gold blank was spectroscopically examined (Figure 1h) in order to exclude the possible contamination of the sample spectrum from an adventitious source.

The 30 °C O 1s spectrum of Chl a, given in Figure 1a, is referenced to the corresponding C 1s binding energy (284.3 eV at 30 °C) to offset the charging effects that are, within experimental errors, the same for both the C 1s and O 1s spectra in the temperature range 30–125 °C. We assign the high binding energy shoulder (533.1 eV at 30 °C) to the oxygen of the Chl a water of hydration. The value 533.1 eV for the O 1s binding energy appears to be indistinguishable from that found for condensed H_2O,[10] or H_2O present in various types of hydrated samples.[11] The main band centered at 531.8 eV appears to be an overlap of spectral contributions attributable to the five oxygen atoms of the Chl a C7 propionic ester, C10 carbomethoxy, and C9 keto groups. This value agrees well with the O 1s value of 531.4 eV reported for the oxygens in sodium benzoate.[12]

The attribution of the high binding energy shoulder at 533.1 eV to the presence of water of hydration is supported by the sequence of XPS Chl a O 1s spectra measured at different temperatures in the 30–250 °C range. No discernible changes are observed as the sample temperature is varied from 30 to 120 °C (Figure 1a–c). At temperatures exceeding 120 °C the high binding energy shoulder begins to diminish and, at 250 °C, this shoulder appears to have vanished quantitatively (Figure 1d–g).