Synthesis of a New Type of Cyclic Peptide: A Bicyclic Nonapeptide

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We report the synthesis of a bicyclic peptide consisting solely of naturally occurring amino acids linked by amide bonds (Figure 1). Metal complexes of such peptides may serve as models for investigating protein–ion interactions. It is known that macrocyclic metal complexes are efficient catalysts and may exhibit unusual stability; the metal ions within macrocyclic complexes sometimes have rare oxidation states, coordination numbers, and geometries.[1] The synthesis of macrobicyclic ligands (cryptates) by Lehn and co-workers[2] has resulted in metal complexes of unprecedented selectivity and stability; the often distorted geometry of metal ion interaction with proteins provides a more complicated example of the atypical behavior observed in macrocyclic and cryptate systems.[3] Thus, bicyclic peptides, topologically similar to the bicyclic cryptates, could serve as useful tools for extending our knowledge of protein–metal ion interactions.

The synthesis of the bicyclic nonapeptide cyclo-(glutamyl[1]-prolyl[2]-glycyl[3]-lysyl[4]-prolyl[5]-glycyl[6])-cyclo-(1γ → 4ϵ)-leucyl[7]-phenylalanyl[8]-alanyl[9] (see Figure 2) involved a 3 + 3 segment condensation to produce a γ-glutamyl linear hexapeptide, followed by the addition of a dipeptide segment and then coupling of the last amino acid. Two separate cyclization steps completed the synthesis, the details of which follow.[4] The protected dipeptide Z-Pro-Gly-O-*t*-Bu[5] was hydrogenated in the presence of 10% Pd/C in 95% ETOH, and the ensuing amine was coupled to Z-Glu-(OBzl)-OH with DCC/HOBt[6] to give, after washing, Z-Glu-(OBzl)-Pro-Gly-O-*t*-Bu, I, as an oil in 100% yield. Hydrogenolysis of the benzyl groups from I, in 95% ETOH containing 1 equiv of HCl, gave the tripeptide hydrochloride, which was dissolved in DMF–H_2O and treated with Na_2CO_3 and *N*-ethoxycarbonylphthalimide[7] (2 equiv of each). Chromatography of the crude product on a column of silica gel (10% MeOH in $CHCl_3$ as eluent) afforded phthalyl-Glu-Pro-Gly-O-*t*-Bu, II, as a homogeneous material: 50%, mp 105–110 °C, $R_f d$ 0.70, $[\alpha]^{23}_D$ −144° (*c* 1, MeOH). Boc-Phe-Ala-NHNHZ, prepared from Boc-Phe-ONp and alanine benzyloxycarbonylhydrazide in the presence of HOBt[8] (91%, mp 84–88 °C, $R_f a$ 0.60, $[\alpha]^{23}_D$ −31° (*c* 1, MeOH), was dissolved in TFA, and the resultant TFA salt was treated with Fmoc-Leu-ONp,[9] HOBt, and DIEA. The product, Fmoc-Leu-Phe-Ala-NHNHZ, was isolated in good yield and purity: 91%, mp 231–233 °C, $R_f a$ 0.50, $[\alpha]^{23}_D$ −50° (*c* 0.5, MeOH). Removal of the Fmoc[10] group with 10% diethylamine in DMF afforded the free amine, which was coupled to II with DCC/HOBt. The product, phthalyl-Glu(Leu-Phe-Ala-NHNHZ)-Pro-Gly-O-*t*-Bu, III, was isolated as a homogeneous material which crystallized from MeOH–ether: 98%, mp 135–138 °C (gel), $R_f a$ 0.70, $[\alpha]^{23}_D$ −88° (*c* 0.3, DMF–MeOH, 1:1). The phthalyl group was removed from III with hydrazine, and the ensuing amine was reacted with Boc-Pro-Gly-ONp[11] to give Boc-Pro-Gly-Glu(Leu-Phe-Ala-NHNHZ)-Pro-Gly-O-*t*-Bu, IV: 85%, mp 130–131 °C (gel), $R_f b$ 0.55, $[\alpha]^{23}_D$ −87° (*c* 0.5, MeOH). IV was dissolved in TFA, and the resultant octapeptide trifluoroacetate was treated with Boc-Lys (*o*-ClZ)-ONo[12] and DIEA to give Boc-Lys(*o*-ClZ)-Pro-Gly-Glu(Leu-Phe-Ala-NHNHZ)-Pro-Gly-OH: 94%, $R_f e$ 0.55, $[\alpha]^{23}_D$ −53° (*c* 1, MeOH). Treatment with excess bis(*p*-nitrophenyl) carbonate[13] gave the nonapeptide active ester, V: 84%, mp 138–141 °C, $[\alpha]^{23}_D$ −45° (*c* 1, DMF). V was dissolved in TFA, the TFA was evaporated in 1 h, and the residue was diluted (10^{-3} M) in DMF, which was then made slightly basic with DIEA. The cyclization was complete within 2 days. The solid residue, after evaporation of DMF, was washed with ethyl acetate and ether to give cyclo-(Lys(*o*-ClZ)-Pro-Gly-Glu-(Leu-Phe-Ala-NHNHZ)-Pro-Gly), VI, in excellent yield and purity: 64%, mp 265–268 °C, $R_f c$ 0.68, $[\alpha]^{23}_D$ +6.4° (*c* 0.5, DMF–H_2O, 1:1). VI was dissolved in 4.5 M HBr in AcOH, and after 4 h the 9-peptide hydrazide hydrobromide was converted to the azide in DMF, with $NaNO_2$ and HCl. The

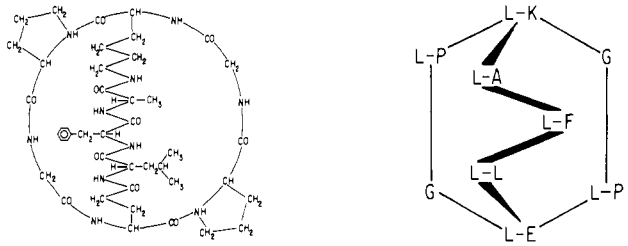

Figure 1. Two representations of the bicyclic nonapeptide (VII).

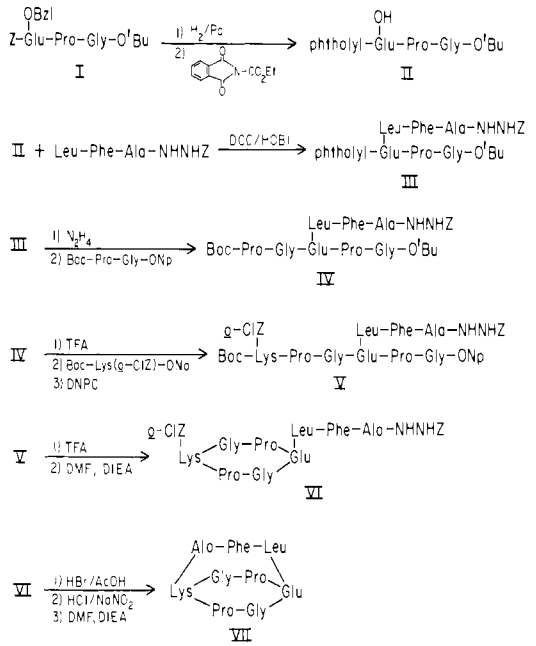

Figure 2. Synthetic scheme for the bicyclic nonapeptide (VII).

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(2) Lehn, J. M. *Acc. Chem. Res.* **1978**, *11*, 49–57.

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(4) Abbreviations: Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; *o*-ClZ, *o*-chlorobenzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DNPC, bis(*p*-nitrophenyl) carbonate; Fmoc, 9-fluorenylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; ONo, *o*-nitrophenyl ester; ONp, *p*-nitrophenyl ester; TFA, trifluoroacetic acid. Thin-layer chromatograms were run in the following solvent systems: (a) $CHCl_3$–CH_3OH, 19:1; (b) $CHCl_3$–CH_3OH, 9:1; (c) $CHCl_3$–CH_3OH, (8:2); (d) ethyl acetate–pyridine–acetic acid–water, 120:20:6:11; (e) ethyl acetate–pyridine–acetic acid–water, 60:20:6:11.

(5) Prepared according to Geiger (Geiger, R.; Volk, A. *Chem. Ber.* **1973**, *106*, 199–205) but isolated as a crystalline solid from ether–hexane rather than an oil, mp 72–73 °C.

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azide (not isolated) was diluted (10^{-3} M) in cold DMF, made slightly basic with DIEA, and stored at 4 °C for 2 days. The product, purified on a column of silica gel (MeOH in $CHCl_3$ as eluent), was isolated as a homogeneous material which crystallized from MeOH: ether as a complex with 2 equiv of NaOAc: 40%, mp 218–219 °C, $[\alpha]^{23}_D$ +40° (*c* 0.5, MeOH). The peptide was desalted on a column of Rexyn I-300 with 50% ETOH as eluent to give VII, cyclo-(Glu[1]-Pro[2]-Gly[3]-Lys[4]-Pro[5]-Gly[6])-cyclo-(1γ → 4ε)-Leu[7]-Phe[8]-Ala[9], which crystallized from MeOH–ether as fine, soft needles: mp 215–216 °C. Mass spectrum $(M + H)^+$ 879 (field desorption and fast atom bombardment). No ninhydrin or fluorescamine reaction with this compound was observed. Satisfactory amino acid and elemental analyses were obtained in all cases for this peptide and the synthetic intermediates.

The above described synthesis resulted in a homogeneous bicyclic peptide in respectable yield. This strategy and methods are being used for the preparation of analogous peptides.

Conformational and metal binding studies are being performed. Initial circular dichroism measurements (in acetonitrile) indicate that the bicyclic peptide binds Zn^{2+} in a 2:1 peptide to Zn^{2+} ratio. Changes in NMR spectra (^{13}C and ^1H) have also occurred upon addition of Zn^{2+}. These changes, as well as those caused by other metal ions, are being investigated.

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Evidence for the Intermediacy of Hexamethyl-1,4-disilabenzene[1]

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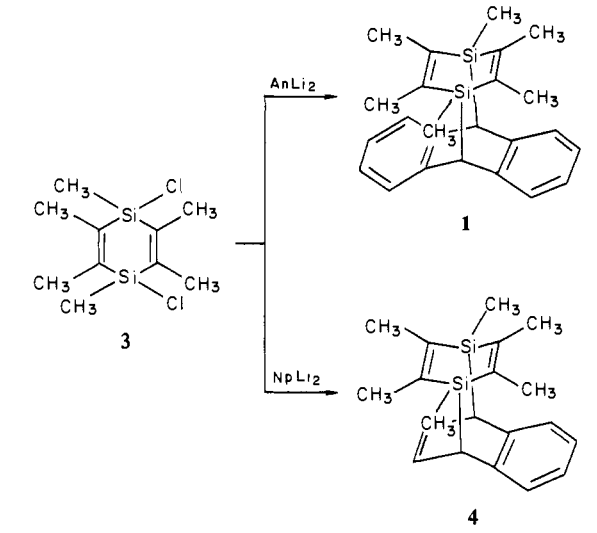
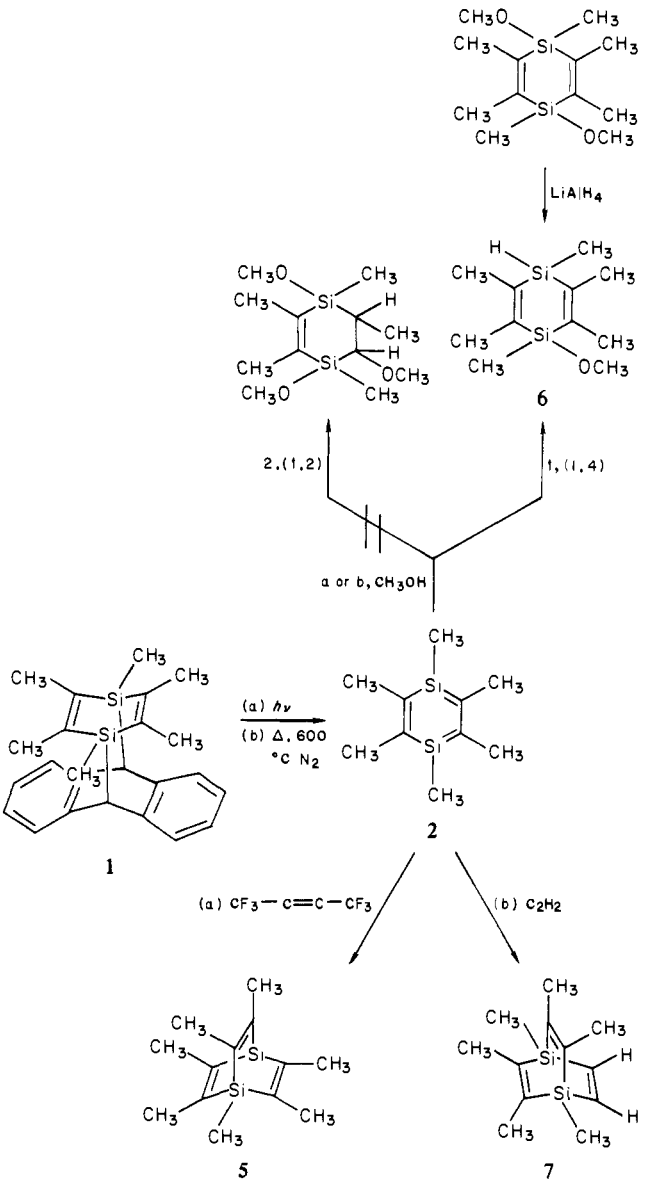
The pioneering work of Barton and co-workers on the generation of silatoluene[2] provided the first example of a potentially aromatic, silicon-containing analogue of benzene. Two other examples of silabenzene intermediates have recently been described.[3] We now report evidence for the existence of the first polysilabenzene, hexamethyl-1,4-disilabenzene (**2**) obtained by photochemical or thermal fragmentation of the precursor **1**.

Reaction of a 1:1 mixture of *cis*- and *trans*-1,4-dichlorohexamethyl-1,4-disilacyclohexa-2,5-diene (**3**) with lithium and either anthracene or naphthalene gave the corresponding bridged adducts **1** and **4** in 25% yields.[4] Both of the adducts fragment under mass spectroscopic conditions in a manner consistent with formation of the disilabenzene; **1** gives a peak with *m/e* 194 (corresponding to the elements of **2**) as the base peak, while **4** gave *m/e* 194 as 27% of the base peak, naphthalene. Subsequent studies were carried out on the anthracene adduct **1**, which is easily recrystallized from THF.[6]



Scheme I



Photolysis of **1** in THF at 254 nm gave anthracene as the only volatile product. When **1** was irradiated in the presence of hexafluoro-2-butyne, anthracene was again isolated but now the disilabarralene derivative **5** was also formed in 88% yield (Scheme

(1) Preliminary results of this work were presented at the 16th Symposium on Organosilicon Chemistry, Midland, MI, June 16, 1982.

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(3) (a) For a review of previous attempts to generate silabenzenes see: Jutzi, P. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 232. (b) 1,4-Di-*tert*-butylsilabenzene: Märkl, G.; Hofmeister, P. *Ibid.* **1979**, *18*, 780. (c) Parent silabenzene: Maier, G.; Mihm, G.; Reisenauer, H. P. *Ibid.* **1980**, *19*, 52. (d) Photoelectron spectra of silabenzene: Solouki, B.; Rosmus, P.; Bock, H.; Maier, G. *Ibid.* **1980**, *19*, 51.

(4) Compound **3** is conveniently synthesized from the corresponding dimethoxy derivative[5] and acetyl chloride.

(5) Atwell, W.; Weyenberg, D. *J. Am. Chem. Soc.* **1968**, *90*, 3848.

(6) Anthracene adduct **1**: mp 246–247.5 °C; NMR (CCl_4, Me_4Si) δ 6.8–6.9 (m, 8 H), 3.5 (s, 2 H), 1.4 (s, 12 H), 0.4 (s, 6 H); mass spectrum *m/e* 372 (8.2), 194 (100), 179 (18).

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