# Studies on Cyclic Peptides. 5. Conformation and Interaction with Small Molecules of Cyclic Hexapeptides Containing Glutamic Acid or Aspartic Acid Residue[1]

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*Received January 5, 1976*

Cyclic hexapeptides containing glutamic acid, aspartic acid, or their esters were synthesized, and the conformation of the cyclic peptides was investigated by NMR spectroscopy. Cyclo-(Gly-X-Gly)$_2$ [X = Glu(OBzl), Glu(OMe), Glu, Asp(OBzl), Asp] were considered to possess $C_2$ conformation in $Me_2SO$ and water in which two glycyl residues preceding the X residue are intramolecularly hydrogen bonded. Substitution on the side chain carboxyl group did not influence the structure of the peptide backbone significantly, and the intramolecular interaction of the peptide backbone with the phenyl ring of benzyl ester was negligible. The effect of additives on the NMR spectra of cyclo-(Gly-X-Gly)$_2$ was examined. The resonance signal of the intramolecularly hydrogen-bonded glycyl peptide protons was shifted noticeably to lower field on addition of guanidine hydrochloride or lithium bromide in $Me_2SO$-$d_6$. The shift could be interpreted almost completely in terms of a conformational change of the peptide backbone. It was suggested that medium effects (polar effects) and increasing association with $Me_2SO$ and with the anion of added salts accompany the conformational change. In water, on the other hand, the NH resonance signals shifted to a higher field on addition of guanidine hydrochloride or lithium bromide. This was ascribed to the salt-induced weakening of the hydration of NH.

Cyclic peptides are functionalized rigid systems and have been used to study the relationship between biological activity and conformation of biomolecules.[3] Synthetic cyclic peptides can also be used as model compounds for proteins including enzyme. Cyclic peptides are small, simple compounds in which several features of proteins can be implanted without producing an end-group effect, because they have neither an N nor a C terminus.

Conformational studies of cyclic peptides have increasing importance,[3] but investigations of cyclic peptides containing glutamic acid or aspartic acid have not yet been carried out, though acidic amino acids play an important role in enzymic reactions.[4] It is advantageous to use these cyclic peptides for the following reasons: (1) by modifying the side chain carboxyl group the effect of the side chain on the conformation of cyclic peptide can be investigated, (2) by introducing a benzyl ester group intramolecular interactions such as side chain–backbone or side chain–side chain interaction may be studied, (3) by modifying the side chain carboxyl group the cyclic peptides are made soluble in various solvents so that the effect of solvent and additives on the conformation of cyclic peptides can be investigated in detail. In the present investigation several cyclic hexapeptides containing acidic amino acid residues or their esters were synthesized, their conformation in solution was investigated by NMR spectroscopy, and their interaction with small molecules was investigated in relation to the conformational properties.

## Experimental Section

**NMR Spectra.** NMR spectra were obtained at 100 MHz with a Varian HA-100 and at 220 MHz with a Varian HR-220 spectrometer. Assignment of resonance signals was made by spin decoupling using a Hewlett-Packard 4204A digital oscillator.

**Materials.** Deuterium compounds were purchased from Fabriqué par CEA-France Service des Molécules Marguées. Inorganic salts were purified by recrystallization. Anhydrous LiBr was obtained by heating at 150 °C for 2 days in vacuo.

**Cyclic Peptides.** Cyclic hexapeptides were synthesized from the respective tripeptide *p*-nitrophenyl esters by cyclodimerization in pyridine under a high dilution.[5,6] Melting points were uncorrected. Thin layer chromatography (TLC) was run on silica gel G plate. Solvent systems for TLC were (A) chloroform–methanol–acetic acid (95:5:3), (B) chloroform–methanol–pyridine (95:5:3), (C) 1-butanol–acetic acid–water (4:1:1), and (D) 1-butanol–acetic acid–water–pyridine (30:6:24:20). Molecular weight was obtained by a cryoscopic determination using the lactam of *cis*-hexahydro-*p*-aminobenzoic acid[7] as a solvent.

**Boc-Glu(OBzl)-Gly-OH (I).** To an aqueous solution of glycine (0.75 g, 0.01 mol) and sodium bicarbonate (1.68 g, 0.02 mol) was added Boc-Glu(OBzl)-ONSu[8] (4.3 g, 0.01 mol) in THF (20 ml). After 1 h the reaction mixture was evaporated to concentrate, acidified with 1 N hydrochloric acid, and then extracted with ethyl acetate. After drying over sodium sulfate the organic layer was evaporated to give an oil, which crystallized on standing after being treated with ether and *n*-hexane. Recrystallization from ethyl acetate–*n*-hexane gave 2.7 g (69%) of I: mp 116–116.5 °C; $[\alpha]^{25}D$ −8.4° (*c* 1.0, EtOH); $R_f^A$ 0.63, $R_f^B$ 0.19, $R_f^C$ 0.82, $R_f^D$ 0.73. Anal. Calcd for $C_{19}H_{26}O_7N_2$: C, 57.86; H, 6.64; N, 7.10. Found: C, 57.45; H, 6.58; N, 7.17.

**Boc-Gly-Glu(OBzl)-Gly-OH (II).** To a solution of I (3.94 g, 0.01 mol) in dioxane (10 ml) was added 4.4 N hydrogen chloride in dioxane (30 ml). The reaction mixture was allowed to stand at room temperature for 30 min. After the solvent had been evaporated to dryness under reduced pressure, the oily residue was dissolved in water (70 ml) containing sodium bicarbonate (2.5 g, 0.03 mol). The solution was extracted with ether twice, and then the THF solution of Boc-Gly-ONSu[9] (2.7 g, 0.01 mol) was added to the aqueous solution. The reaction mixture was treated as described for the preparation of I. The residual oil was treated with *n*-hexane and gave a white gel, yield 2.50 g (66%), $R_f^A$ 0.35.

**Boc-Gly-Glu(OBzl)-Gly-ONp (III).** II (1.4 g, 3 mmol) and *p*-nitrophenol (0.42 g, 3 mmol) were dissolved in THF (50 ml) at 0 °C. To the solution DCC (0.62 g, 3 mmol) was added. It was kept at 0 °C for 2 h and then overnight at room temperature. The precipitated dicyclohexylurea was removed by filtration and the filtrate was concentrated. Crystallization occurred upon the addition of 2-propanol. The slightly yellow precipitate was recrystallized from 2-propanol: yield 1.29 g (75%); mp 81–84 °C; $[\alpha]^{25}D$ −10.8° (*c* 1.0, DMF); $R_f^A$ 0.73, $R_f^B$ 0.74, $R_f^C$ 0.83, $R_f^D$ 0.81. Anal. Calcd for $C_{27}H_{32}O_{10}N_4$: C, 56.64; H, 5.63; N, 9.79. Found: C, 56.60; H, 5.62; N, 9.58.

**Cyclo-(Gly-Glu(OBzl)-Gly)$_2$ (IV).** To a dioxane solution of III (614 mg, 1 mmol) was added 4 N hydrogen chloride in dioxane (3 ml). After 30 min a white precipitate was filtered and dried over $P_2O_5$ in vacuo, yield of HCl·Gly-Glu(OBzl)-Gly-ONp 468 mg (87%), mp 162–163 °C. It was dissolved in DMF (10 ml) containing glacial acetic acid (0.1 ml). The solution was added drop by drop into pyridine (350 ml) at 60 °C over a 3-h period. The reaction mixture was kept at room temperature overnight. After the solvent had been evaporated off; methanol (100 ml) was added to the residue to give a white solid (252 mg, 92%). Recrystallization from DMF–methanol gave 202 mg (74%) of IV: mp 298 °C; $[\alpha]^{25}D$ −29.2° (*c* 1.0, DMF); $R_f^B$ 0.08, $R_f^C$ 0.70, $R_f^D$ 0.73; mol wt 623 ± 75 (theory 666.67). Anal. Calcd for $C_{32}H_{38}O_{10}N_6$: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.41; H, 5.70; N, 12.47. The same product is obtainable from the cyclodimerization of Boc-Glu(OBzl)-Gly-Gly-ONp [mp 112–114 °C, $[\alpha]^{25}D$ −1.5° (*c* 1.0, DMF)]. However, the yield of this reaction was as low as 40%.

**Table I. Peptide Proton Resonances and Conformation Parameters of Cyclic Hexapeptides[a]**

| Cyclic hexapeptide | Solvent | $Gly_I$ | | | | $Gly_{II}$ | | | | X | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\delta^b$ | $d\delta^c/dT$ | $J(\Sigma)^d$ | $\phi^e$ | $\delta^b$ | $d\delta^c/dT$ | $J(\Sigma)^d$ | $\phi^e$ | $\delta^b$ | $d\delta^c/dT$ | $J^f$ | $\phi^e$ |
| IV | $Me_2SO$ | 7.47 | 0.0001 | 9.5 | −150 | 8.40 | 0.0035 | 11.6 | +70 | 8.45 | 0.0047 | 6.5 | −80 |
| VI | $Me_2SO$ | 7.51 | 0.0002 | 9.2 | −150 | 8.29 | 0.0052 | 11.0 | +60 | 8.35 | 0.0047 | 6.0 | −75 |
| VI | $H_2O$ | 6.65 | 0.0023 | 10.0 | −140 | 7.33 | 0.0068 | 11.5 | +70 | 7.28 | 0.0074 | 6.5 | −80 |
| V | $Me_2SO$ | 7.54 | 0.0004 | 10.2 | −140 | 8.37 | 0.0047 | 10.6 | +60 | 8.40 | 0.0039 | 6.5 | −80 |
| V | $H_2O$ | 6.64 | 0.0022 | 10.0 | −140 | 7.34 | 0.0065 | 12.0 | +70 | 7.28 | 0.0071 | 6.5 | −80 |
| X | $Me_2SO$ | 7.65 | 0.0001 | 9.0 | −150 | 8.34 | 0.0057 | 11.0 | +60 | 8.68 | 0.0043 | 6.0 | −75 |
| XI | $Me_2SO$ | 7.57 | 0.0006 | 9.2 | −150 | 8.27 | 0.0056 | 10.6 | +60 | 8.61 | 0.0068 | 6.0 | −75 |

[a] Cyclic hexapeptides investigated are represented as cyclo-($Gly_I$-X-$Gly_{II}$)$_2$. [b] Tetramethylsilane in $Me_2SO$-$d_6$ and *tert*-butyl alcohol in water as internal references. [c] Temperature coefficient, ppm to a higher field per degree. [d] Sum of H–$C^\alpha$–N–H coupling from NH resonance. [e] Conformational angle determined from $J$ value and CPK model. [f] From $C^\alpha H$ and NH resonance in $Me_2SO$-$d_6$ and from NH resonance in water.

**Cyclo-(Gly-Glu-Gly)$_2$ (V).** To IV (203 mg, 0.3 mmol) was added anhydrous HF (1 ml) in the presence fo anisole (0.18 ml). After standing at 0 °C for 1 h HF was removed. The residue was solidified with ether and dried over KOH in vacuo. The product V was recrystallized from water–methanol–ether: yield 107 mg (73%); mp 246 °C; $[\alpha]^{25}D$ −17.1° (*c* 1.0, DMF); $R_f^C$ 0.30, $R_f^D$ 0.59. Anal. Calcd for $C_{18}H_{26}O_{10}N_6 \cdot 2H_2O$: C, 41.37; H, 5.79; N, 16.09. Found: C, 41.11; H, 5.38; N, 16.01.

**Cyclo-(Gly-Glu(OMe)-Gly)$_2$ (VI).** Boc-Glu(OMe)-OH[10] (11.8 g, 45 mmol), *N*-hydroxysuccinimide (5.2 g, 45 mmol), and DCC (9.3 g, 45 mmol) were reacted in ethyl acetate (150 ml) at 0 °C for 1 h and the reaction mixture was allowed to stand at room temperature overnight. After precipitated dicyclohexylurea had been filtered, the solution was evaporated. The residual oil ($R_f^A$ 0.53) was dissolved in THF (60 ml) and was added to the aqueous solution (60 ml) of glycylglycine (5.3 g, 40 mmol) and sodium bicarbonate (6.6 g, 80 mmol). The reaction mixture was allowed to stand at room temperature for 1 h. After THF had been evaporated, the solution was extracted with ether, acidified with 1 N hydrochloric acid, and then extracted with ethyl acetate. The organic layer dried over sodium sulfate was concentrated to a colorless syrup (7.5 g, $R_f^A$ 0.09). The residue was treated with *p*-nitrophenol (2.8 g, 20 mmol) and DCC (4.1 g, 20 mmol) in ethyl acetate (50 ml) at 0 °C. After 24 h dicyclohexylurea was removed by filtration and the filtrate was evaporated to give a slightly yellow syrup (9.8 g, $R_f^A$ 0.22). It was washed with ether and *n*-hexane. Hydrogen chloride (4 N) in dioxane (40 ml) was added to the oil. After 20 min the solvent was evaporated to give a white solid. It was washed twice with ethyl acetate and dissolved in DMF (50 ml) containing glacial acetic acid (0.5 ml). Cyclization was accomplished in pyridine (500 ml) as described above for the preparation of IV. Recrystallization from hot water–methanol gave 245 mg of VI: mp 320 °C; $[\alpha]^{25}D$ −15.6° (*c* 0.5, $H_2O$); $R_f^C$ 0.21, $R_f^D$ 0.56; mol wt 537 ± 34 (theory 514.49). Anal. Calcd for $C_{20}H_{30}O_{10}N_6$: C, 46.69; H, 5.88; N, 16.34. Found: C, 46.46; H, 5.76; N, 16.27.

**H-Asp(OBzl)-Gly-OH (VII).** To an aqueous solution (20 ml) of glycine (0.75 g, 10 mmol) and sodium bicarbonate (1.68 g, 20 mmol) was added slowly Boc-Asp(OBzl)-ONSu[11] (4.2 g, 10 mmol) in THF (20 ml) at room temperature. After the solution was stirred for 30 min the reaction mixture was treated as described above for the preparation of I. Hydrogen chloride (4 N) in dioxane (30 ml) was added to the oily product (3.8 g). After standing at room temperature for 30 min the solvent was evaporated. The residue was taken up in ethanol (10 ml) and neutralized by triethylamine. The white mass was recrystallized from water–acetone. The yield was 2.1 g (75%): mp 170–171 °C; $[\alpha]^{25}D$ +51.5° (*c* 1.0, $H_2O$); $R_f^C$ 0.46, $R_f^D$ 0.63. Anal. Calcd for $C_{13}H_{16}O_5N_2$: C, 55.71; H, 5.75; N, 10.00. Found: C, 55.79; H, 5.77; N, 10.10.

**Boc-Gly-Asp(OBzl)-Gly-OH (VIII).** An ethanolic solution (40 ml) of Boc-Gly-ONSu[9] (1.36 g, 5 mmol) was added slowly to the aqueous solution of VII (1.40 g, 5 mmol) and sodium bicarbonate (0.80 g, 10 mmol). After 20 h the solution was treated as described above for the preparation of II. Recrystallization from ethyl acetate–ether gave white crystals: yield 1.45 g (66%); mp 113.5–114 °C; $[\alpha]^{25}D$ −18.3° (*c* 1.0, EtOH); $R_f^A$ 0.50, $R_f^B$ 0.13, $R_f^C$ 0.75, $R_f^D$ 0.74. Anal. Calcd for $C_{20}H_{27}O_8N_3$: C, 54.91; H, 6.22; N, 9.61. Found: C, 54.57; H, 6.23; N, 9.54.

**Boc-Gly-Asp(OBzl)-Gly-ONp (IX).** This compound was synthesized from VIII (1.75 g, 4 mmol), *p*-nitrophenol (0.56 g, 4 mmol), and DCC (0.82 g, 4 mmol) as described above for the preparation of III. The product was recrystallized from ethyl acetate–*n*-hexane: yield 1.90 g (85%); mp 83–85 °C; $[\alpha]^{25}D$ −21.3° (*c* 1.0, DMF); $R_f^A$ 0.77, $R_f^B$ 0.83, $R_f^C$ 0.84, $R_f^D$ 0.82. Anal. Calcd for $C_{26}H_{30}O_{10}N_4$: C, 55.91; H, 5.14; N, 10.03. Found: C, 55.94; H, 5.69; N, 10.08.

**Cyclo-(Gly-Asp(OBzl)-Gly)$_2$ (X).** Hydrogen chloride (4 N) in dioxane (3.6 ml) was added to IX (647 mg, 1.2 mmol). After 30 min the white precipitate was filtered and dried in vacuo (465 mg, mp 166–168 °C). The tripeptide ester was cyclized in pyridine (350 ml) as described above for the preparation of IV. A white solid appeared upon the addition of ethanol to the residual oil which was recovered by evaporation. It was recrystallized from DMF–water–ether. The yield was 135 mg (40%): mp 256 °C; $[\alpha]^{25}D$ −33.1° (*c* 1.0, DMF); mol wt 644 ± 31 (theory 638.62); $R_f^B$ 0.05, $R_f^C$ 0.64, $R_f^D$ 0.65. Anal. Calcd for $C_{34}H_{34}O_{10}N_6$: C, 56.42; H, 5.37; N, 13.16. Found: C, 56.46; H, 5.69; N, 13.11.

**Cyclo-(Gly-Asp-Gly)$_2$ (XI).** X (220 mg, 0.35 mmol) was allowed to react with anhydrous HF and treated as described above for the preparation of V. Recrystallization from water–ethanol–ether gave 120 mg (72%) of XI, mp 270 °C. Anal. Calcd for $C_{16}H_{22}O_{10}N_6 \cdot 2H_2O$: C, 38.86; H, 5.30; N, 16.99. Found: C, 38.51; H, 5.11; N, 17.07.

## Results and Discussion

**Conformation of Peptide Backbone and Side Chain.** Either in $Me_2SO$-$d_6$ or in water resonance signals corresponding to three amino acid residues were observed for all of the cyclic hexapeptides studied, which have six amino acid residues. It is therefore very likely that the cyclic hexapeptides possess $C_2$ symmetry in these solvents on the NMR time scale. It would be convenient to number the amino acid residues in the cyclic hexapeptides, when necessary, as cyclo-($Gly_I$-X-$Gly_{II}$)$_2$ [X = Glu(OBzl), Glu(OMe), Glu, Asp(OBzl), or Asp]. Parameters concerning the resonance signals for the peptide proton (NH) of the cyclic hexapeptides are described in Table I. There is very little change in chemical shift and temperature coefficient of the resonance signals for the peptide protons of the individual amino acid residues when X is varied. In $Me_2SO$-$d_6$ a resonance signal for one of the two types of glycyl peptide protons is located at higher magnetic field ($\delta$ 7.47–7.65 ppm) with a small temperature coefficient (0.0001–0.0006 ppm/deg) (represented as $Gly_I$ in Table I; see also the text), while those for the other glycyl peptide protons (represented as $Gly_{II}$ in Table I; see also the text) and two X peptide protons are located at lower magnetic fields ($\delta$ 8.27–8.40 and 8.35–8.68 ppm, respectively) with a large temperature coefficient (0.0035–0.0074 ppm/deg). This type of peptide proton resonance has been reported to be characteristic of the $C_2$-symmetric conformation consisting of two $\beta$ turns with two glycyl peptide protons intramolecularly hydrogen bonded, and the other two glycyl peptide protons exposed to solvent on the NMR time scale.[12] This was the case for the five cyclic hexapeptides investigated here.

It would be valuable to definitively assign the conformational positions of the X residues in the cyclic structure and

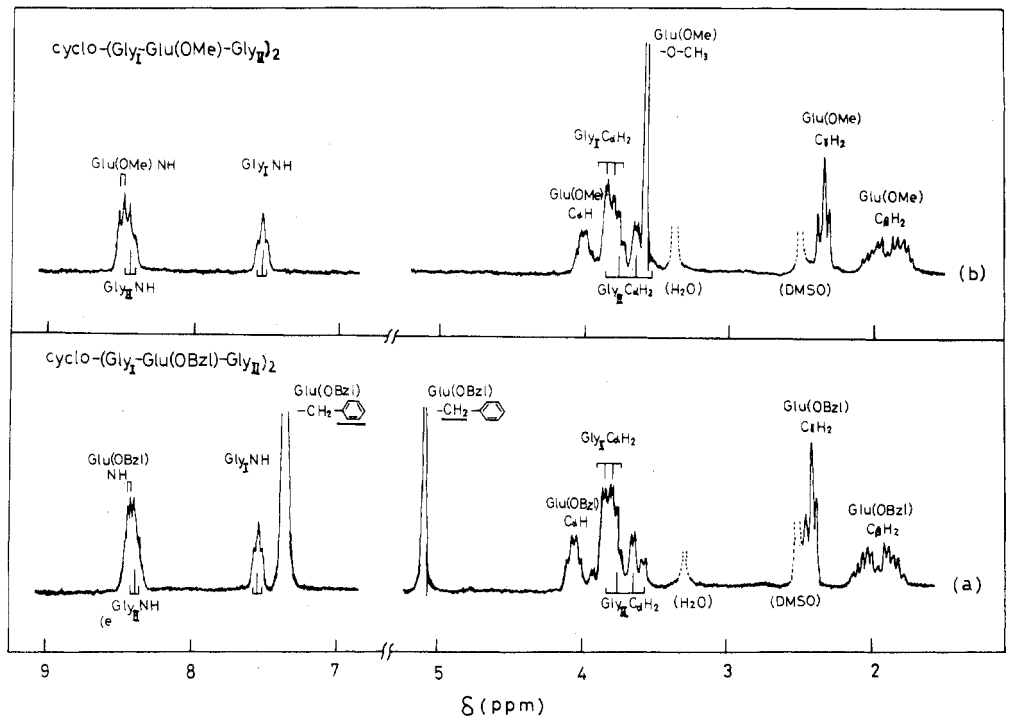


**Figure 1.** 220-MHz NMR spectra of IV (a) and VI (b) in $Me_2SO$-$d_6$.

to decide what glycine residues are intramolecularly hydrogen bonded. Spin-decoupling experiments revealed that the C$^\alpha$H proton of the X residue is vicinal to the NH proton in the lower field. Therefore, the X residues carrying a bulky side chain are considered to take the corner positions of the $\beta$ turn, as has been reported by Kopple et al.[12-14] for mono- or 1,4-disubstituted cyclic hexapeptides. In other words, the extended parts of the cyclic structure are occupied by glycine residues. There are two types of $\beta$ turn, depending on whether the residues preceding or succeeding the X residues are transannularly hydrogen bonded (type A or B according to Pease et al.[15]). Recent investigations using $^1H$[12-14] or $^{13}C$[15] NMR spectroscopy on the selectively deuterated cyclic hexapeptides showed that the type A $\beta$ turn is a likely conformation for cyclo-(Gly-X-Gly)$_2$, where X = Pro, Tyr, and so on. After these investigations it was considered that two glycine residues preceding the X residues, that is Gly$_I$ in the present representation, are transannularly hydrogen bonded in the present cyclic hexapeptides.

Substitution on the side chain carboxyl group [X = Glu, Glu(OMe), or Glu(OBzl)] did not influence significantly the conformation of the cyclic hexapeptides in $Me_2SO$-$d_6$, as is clearly seen from the $\phi$ values shown in Table I. $\phi$ values were calculated using the Karplus-type equation which relates coupling constant $J$ with dihedral angle $\theta$ of H-N-C$^\alpha$-H.[14] It is also seen that the length of the side chain did not influence the conformation of the cyclic hexapeptides in $Me_2SO$-$d_6$. This is evident from the comparison of $\phi$ angles of X and XI with those of IV and V, respectively.

Comparison of IV and VI will give precise information on the conformation of the side chain. Magnetic anisotropy of the benzene ring of the benzyl ester could cause an upfield shift of the NMR spectrum of the peptide backbone,[16] if the benzyl group of the side chain stacks over the peptide backbone. In Figure 1, however, there is little difference in chemical shift and H-N-C$^\alpha$-H coupling constants of the two spectra, except for the C$^\alpha$H, C$^\beta$H$_2$, and C$^\gamma$H$_2$ resonance signals of Glu(OBzl) which appear at slightly lower magnetic field than those of Glu(OMe). This difference could have been caused by the more electron-withdrawing benzyl group. The above result indicates the conformational similarity of the two cyclic hexapeptides and the absence of intramolecular interaction between the peptide backbone and the benzene ring of the side chain. This is also evident from the similarity of the chemical shifts of the phenyl protons of IV and II[17] in $Me_2SO$-$d_6$.

A large splitting of the C$^\alpha$H$_2$ resonance of the Gly$_{II}$ residue was observed: 0.19 ppm for IV and 0.21 ppm for VI. This sort of splitting has usually been interpreted in terms of a different environment of the two protons.[14] On the other hand, a small splitting of the C$^\alpha$H$_2$ resonance was observed for the Gly$_I$ residue: 0.08 ppm for IV and 0.10 ppm for VI. This small splitting indicates that the two protons are in a similar environment. It is also evident above that there is little difference in the splittings of the C$^\alpha$H$_2$ resonances of IV and VI. The splitting of the Gly$_{II}$-C$^\alpha$H$_2$ signal of IV (0.19 ppm) is only slightly larger than that of the Gly$_I$-C$^\alpha$H$_2$ signal (0.08 ppm). These experimental findings confirm that interactions of the phenyl ring with the peptide backbone are absent. If they interacted, the methylene splitting of IV would have been much larger.[13]

In aromatic cyclic dipeptides the aromatic side chain is frequently folded onto the main chain amide,[18-24] while for larger cyclic peptides such folding is rare. Kopple et al.[13] have reported that the tyrosine side chain of cyclo-(Gly-Gly-His-Gly-Gly-Tyr) and cyclo-(Gly-Gly-Gly-Gly-Gly-Tyr) might interact with the peptide backbone in $Me_2SO$. Walter et al.[25,26] have reported that the aromatic side chains of adjacent tyrosine and phenylalanine residues of lysine vasopressin and arginine vasopressin stack with each other so that the ring current effect caused an anomalous downfield shift of the phenylalanyl NH resonance in $Me_2SO$. In order for an aromatic group to associate intramolecularly with a peptide bond, a number of requirements need be fulfilled. In this respect, Kopple's observation[19] that in a homologous series of cyclo-(Gly-X) where X is phenylglycine, phenylalanine, or homophenylalanine Gly-C$^\alpha$H$_2$ is shielded by the nearby aromatic ring only in cyclo-(Gly-Phe) is very suggestive. The intramolecular aromatic-amide interaction can be achieved only when the optimum alignment of the relevant groups is attained at the expense of unfavorable side chain orientations.

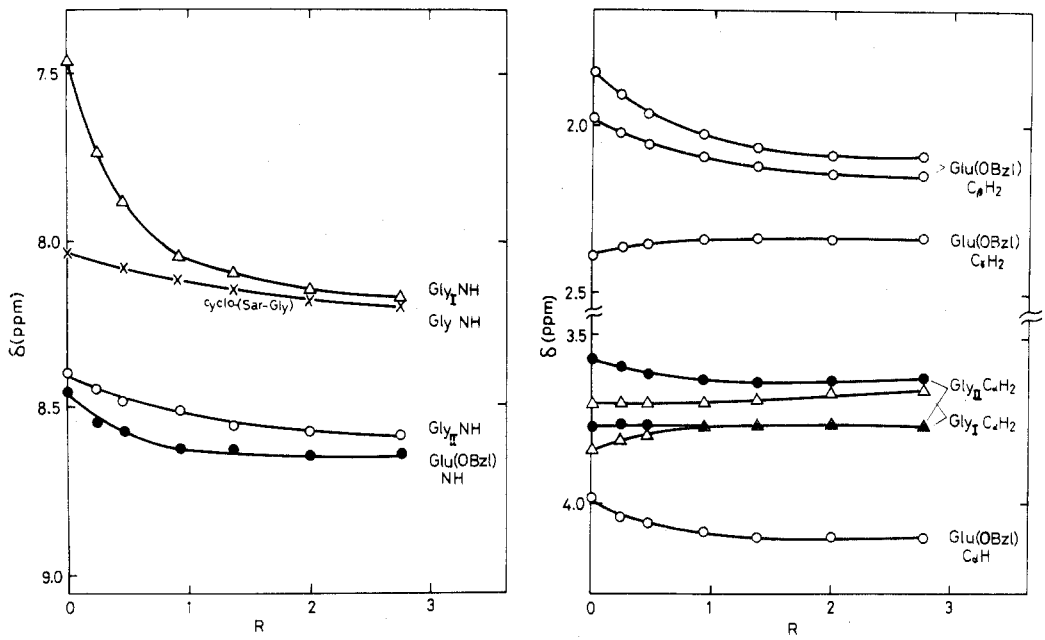


**Figure 2.** The shift of resonance signals of IV with the addition of guanidine hydrochloride in $Me_2SO$-$d_6$. Concentrations of IV and cyclo-(Sar-Gly) were 44 and 48 mg/ml, respectively. *R*: moles of guanidine hydrochloride per unit mole of peptide bond of IV.

The failure to maintain the folded conformation of IV and X could be due mainly to the unsatisfactory fulfillment of the above requirement.

Intermolecular aromatic–amide interactions were found to be weak, too. On addition of benzene-$d_6$ up to 50% to a solution of cyclo-(Gly-X-Gly)$_2$ in $Me_2SO$-$d_6$, all resonance signals shifted only slightly to lower field. The absence of interaction with benzene may be attributable to the polarity of solvent. Interactions of the cyclic peptide with strongly polar $Me_2SO$ destroys the weak amide–aromatic compound interactions. In a previous investigation[16] the intermolecular interaction between cyclic peptides and benzene was observed only in a less polar solvent such as $CDCl_3$. Therefore, the absence of intramolecular aromatic–amide interactions in IV and X could be partly ascribed to the breakdown of the weak interactions, if any does occur, by highly dipolar $Me_2SO$.

The NMR pattern of peptide protons of V and VI observed in $Me_2SO$ was also observed in water. Two solvent-shielded glycyl peptide protons (Gly$_I$-NH) and four solvent-exposed peptide protons (Gly$_I$-NH and X-NH) were present. The temperature coefficient for Gly$_I$-NH signal in water (0.002 ppm/deg) was larger than that in $Me_2SO$-$d_6$ (0.0002–0.0004 ppm/deg). So the Gly$_I$-NH proton must be more solvent exposed in water than in $Me_2SO$-$d_6$. *J* values ($\phi$ angles) were less sensitive to the nature of solvent (Table I). As far as the *J* values concern, V and VI assume the same conformation either in water or in $Me_2SO$-$d_6$. It can be considered that the backbone of cyclic peptides undergoes some segmental motion depending on the environment (solvent, temperature). The oscillating motion should bring the internal peptide proton in contact with solvent, a situation which is reflected in a larger temperature coefficient in water than in $Me_2SO$. On the other hand, this sort of oscillating segmental motion does not influence the time-averaged dihedral angle. This is the reason why the *J* values were almost insensitive to the nature of solvent, whereas the temperature coefficients were sensitive. This speculation might be supported by a $T_1$ study using $^{13}C$ NMR spectroscopy,[27] which is now underway.

In trifluoroacetic acid (TFA), only two NH resonance signals were observed with IV. The benzyl glutamyl peptide protons resonated at 8.06 ppm and the glycyl peptide protons resonated at 7.95 ppm. Their intensity ratio was 1:2. The higher field resonance signal for the glycyl peptide protons observed in $Me_2SO$ was not observed in TFA. That only one resonance signal was present for the glycyl peptide protons could be due to fortuitous overlapping of two resonance signals, one of which is upfield in $Me_2SO$ but shifted downfield in TFA, and the other downfield in $Me_2SO$ but shifted upfield in TFA. These changes might have been caused by the change of the type of hydrogen bonding from $Me_2SO$···HN to TFA···HN, $Me_2SO$ being a stronger proton acceptor in hydrogen bonding than TFA.[28] The same pattern of solvent effect on the chemical shifts of peptide proton signals has been observed by Kopple et al. for cyclo-[Gly($d_2$)-Tyr-Gly($d_2$)]$_2$ and cyclo-(Gly-Leu-Gly)$_2$.[14] These NMR changes may be interpreted as well in terms of a conformational change of the peptide backbone. In fact, the resonance signal of benzyl methylene protons was a small doublet (2 Hz at 220 MHz) in TFA. This is not caused by the decomposition of benzyl ester group, because NMR spectra were recorded soon after the solution was prepared.[29] These events are quite different from those observed in $Me_2SO$-$d_6$. To explain all the phenomena uniformly, one should consider a conformational change. Some other conformation of IV may be favored in TFA than the $C_2$-symmetric one in $Me_2SO$ or water. A number of cyclic hexapeptides not containing imino acid have been synthesized and their conformation in solution has been investigated. The $C_2$ conformation stated above has been shown to prevail in these cyclic hexapeptides.[30] The unusual conformation of IV in TFA observed here is therefore an interesting phenomenon.

To summarize, cyclic hexapeptides containing glutamic or aspartic acid residue were synthesized and their conformation was first investigated in various solvents. They have the $C_2$-symmetric conformation with two internally hydrogen-bonded glycine residues in $Me_2SO$ and water. The nature of the side chains had little effect on the conformation. A different conformation was observed for IV in TFA.

**Effect of Additives.** The effect of additives on the NMR spectra of cyclo-(Gly-X-Gly)$_2$ was examined in $Me_2SO$-$d_6$ and water. Figure 2 shows the shift of the NMR signals of IV caused by the addition of guanidine hydrochloride in $Me_2SO$-$d_6$. A downfield shift was observed for the NH, glutamyl $C^\alpha H$, Gly$_{II}$-$C^\alpha H_2$, and glutamyl $C^\beta H_2$ resonance signals, while an upfield shift was observed for the glutamyl $C^\gamma H_2$ and

**Table II. Shift of NMR Signals of Cyclic Hexapeptides[a] Induced by Additives**

| Additives | Cyclic hexapeptide | Solvent | Concn,[b] mg/ml | $R$[c] | Δδ,[d] ppm: NH $Gly_I$ | NH $Gly_{II}$ | NH X | β X | γ X | α X | α $Gly_I$ | α $Gly_{II}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LiBr | IV | $Me_2SO$ | 55 | 2 | 0.50 | 0.06 | 0.12 | 0.24<br>0.24 | 0 | 0.14 | | |
| Gu·HCl | IV | $Me_2SO$ | 55 | 2 | 0.67 | 0.13 | 0.16 | 0.25<br>0.16 | −0.06 | 0.10 | −0.04<br>−0.07 | 0<br>0.06 |
| Gu·$HNO_3$ | IV | $Me_2SO$ | 55 | 2 | 0.13 | 0.01 | 0.01 | 0 | 0 | 0 | 0 | 0 |
| LiI | IV | $Me_2SO$ | 55 | 2 | 0.06 | −0.02 | 0.01 | | | | | |
| LiSCN | IV | $Me_2SO$ | 55 | 2 | 0.13 | 0.00 | 0.06 | | | | | |
| $LiClO_4$ | IV | $Me_2SO$ | 55 | 2 | 0.06 | −0.03 | 0 | | | | | |
| $LiNO_3$ | IV | $Me_2SO$ | 55 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LiBr | VI | $Me_2SO$ | 44 | 2 | 0.62 | 0.11 | 0.14 | | | | | |
| LiBr | VI | $H_2O$ | 22 | 10 | −0.02 | −0.21 | −0.17 | | | | | |
| Gu·HCl | VI | $H_2O$ | 22 | 10 | 0.04 | −0.04 | −0.04 | | | | | |
| Gu·HCl | VI | $Me_2SO$ | 44 | 2 | 0.57 | 0.20 | 0.23 | | | | | |
| LiBr | V | $Me_2SO$ | 33 | 2 | 0.45 | 0.07 | 0.14 | 0.18<br>0.16 | −0.01 | 0.13 | | |
| Gu·HCl | V | $Me_2SO$ | 33 | 2 | 0.56 | 0.21 | 0.23 | 0.21<br>0.20 | 0 | 0.10 | | |
| LiBr | X | $Me_2SO$ | 33 | 2 | 0.71 | 0.18 | −0.03 | 0.26<br>0.21 | | 0.23 | | |
| Gu·HCl | X | $Me_2SO$ | 33 | 2 | 1.09 | 0.46 | 0.20 | 0.25<br>0.17 | | 0.22 | 0<br>−0.01 | 0<br>0.10 |

[a] Cyclic hexapeptides investigated are represented as cyclo-($Gly_I$-X-$Gly_{II}$)$_2$. [b] Concentration of cyclic hexapeptide. [c] Moles of additives per unit mole of peptide bond of cyclo-(Gly-X-Gly)$_2$. [d] Shift of NMR signals with the addition of the additives. Minus sign designates the shift to an upper field.

$Gly_I$-$C^\alpha H_2$ resonance signals. No shift was observed for benzyl resonance signals. The shift was most marked were $R$ (moles of additive per unit mole of peptide bond) was smaller than 1, and became least marked where $R$ exceeded 2. The effect of lithium bromide was quite similar to that of guanidine hydrochloride. It should be noted here that the downfield shift of the resonance signal of the intramolecularly hydrogen-bonded glycyl peptide protons ($Gly_I$-NH) appearing at upper field was far greater than that of the other two resonance signals due to the intermolecularly hydrogen-bonded peptide protons ($Gly_{II}$-NH, X-NH) appearing at a lower field. The effect of guanidine hydrochloride and lithium bromide on the NMR spectra of V, VI, and X in $Me_2SO$-$d_6$ was investigated as well. In all the case, the effect of additives was similar; the experimental results are summarized in Table II.

The large downfield shift of the $Gly_I$-NH protons induced by the salts could be explained as follows: (1) either the cation or the anion of the added salts interacts with the peptide bond of the cyclic hexapeptides, or the anion interacts with the peptide proton;[31] (2) consequently the backbone conformation of the cyclic hexapeptides is changed, so that the peptide protons emerge out of the plane of the cyclic hexapeptide and become more exposed to solvent; (3) concurrently the intramolecular hydrogen bonding is weakened; (4) the peptide protons suffer the polar effect of solvent; and (5) the exposed peptide NH associates with solvent and with the anion of the added salts.[31] The effect of added salts seemed to be complex, and any one of the reasons 1–5 alone does not seem to fully explain the experimental findings as described below.

In cyclic hexapeptides containing two L-Pro-D-Phe sequences where the internal peptide protons are almost completely shielded from solvent, the chemical shift of the internal peptide proton signal has been reported to be dependent on the strength of hydrogen bonding.[32] However, in the present cyclo-($Gly_I$-X-$Gly_{II}$)$_2$, the shielding of the $Gly_I$-NH by solvent is not complete and a rapid dynamic equilibrium between an intramolecular hydrogen bonding and a hydrogen bonding with solvent may have been established. For these cyclic hexapeptides it would be unsuitable to ascribe the downfield shift entirely to the weakening of the intramolecular hydrogen bonding.

Cation binding to the carbonyl oxygen and anion binding to the peptide nitrogen may take place as the result of salt addition. Since the NMR shift changes shown in Table II depend on the anion used when various lithium salts were added, the salt effect appears to be associated with the anion. However, binding of the anion to the peptide nitrogen may increase the electron density of the latter and might therefore bring about an upfield shift of the peptide proton signal. However, this was not observed. Therefore we have to postulate a significant change in the environment of the peptide protons that overcomes the effect of the anion binding and causes the peptide protons to shift downfield.

Since the anion of the added salt is not well solvated in $Me_2SO$, it will tend to associate with the peptide proton. This may also induce a change in conformational distribution.[31]

The conformational change of the cyclic hexapeptides induced by added salts is evident in terms of the change of $\phi$ angles calculated on the basis of the coupling constant $J_{H\text{-}N\text{-}C^\alpha\text{-}H}$, which are shown in Table III. As the result of the conformational change, the $Gly_I$-NH protons, which originally were in the plane of the cyclic hexapeptide, now protrude from the plane to some extent. It has been reported that the resonance signal of the intramolecularly hydrogen-bonded peptide protons appears at higher field due to the magnetic anisotropic effect of the peptide bond residing at the corner of the $\beta$ turn.[33] On the basis of the $\phi$ values in the presence of the added salts and using the anisotropic magnetic susceptibility of formamide given by Tigelaar and Flygare,[34] the extent of shielding of the $Gly_I$-NH protons was calculated. Calculations shown in Table III revealed that the change of the magnetic anisotropy caused by the conformational change can explain about one-third the amount of the downfield shift.

The $Gly_I$-NH protons that become more exposed to solvent

**Table III. Shielding of Peptide Protons and the Conformational Change of IV[a] in $Me_2SO$**

| | $Gly_I$-NH | | | $Gly_{II}$-NH | | | Glu(OBzl)-NH | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $R$[b] | $\delta$, ppm | $J$[c], Hz | $\phi$, deg | $\delta$, ppm | $J$,[c] Hz | $\phi$, deg | $\delta$, ppm | $J$, Hz[d] | $\phi$, deg | $\Delta\delta$,[e] ppm |
| 0 | 7.47 | $\Sigma = 9.5$ | −150 | 8.40 | $\Sigma = 11.6$ | +70 | 8.45 | 6.5 | −80 | 0.68 |
| 2 | 8.14 | $\Sigma = 11.0$ | −130 | 8.53 | $\Sigma = 11.0$ | +60 | 8.60 | 8.0 | −90 | 0.46 |

[a] IV represents cyclo($Gly_I$-Glu(OBzl)-$Gly_{II}$)$_2$. [b] Moles of guanidine hydrochloride per unit mole of peptide bond of cyclo-(Gly-Glu(OBzl)-Gly)$_2$. [c] Sum of H–C$^\alpha$–N–H coupling constant from C$^\alpha$H and NH signals. [d] H–C$^\alpha$–N–H coupling constant from C$^\alpha$H and NH signals. [e] Calculated shielding of $Gly_I$-NH with respect to $Gly_{II}$-NH.

as a result of the conformational change should be subject to the polar effect of solvent. It has been reported that the downfield shift due to the polar effect is proportional to the square of an electric field.[35] If the value (ca. 0.15 ppm) observed for the resonance signal of the solvent-exposed peptide proton of cyclo-(Gly-Sar) (see Figure 2) is taken as a measure of the polar effect, the sum of the conformational and the polar effects can explain more than half of the downfield shift.

The rest of the shift changes (ca. 0.3 ppm) could have been caused by the increasing extent (for the formerly intramolecularly hydrogen-bonded $Gly_I$-NH protons) of hydrogen bonding with solvent. The exposed NH will tend to associate also with the anion of the added salts which is not well solvated in $Me_2SO$. Insofar as the immediate interaction of NH with anion resembles hydrogen bonding, it will give rise to a downfield shift.[31] These considerations receive support from the experimental results shown in Figure 3. Firstly the temperature coefficient for the resonance signal of $Gly_I$-NH increased from 0.0001 ppm/deg to 0.003 ppm/deg with the addition of guanidine hydrochloride ($R$ = 2), while those of $Gly_{II}$-NH and glutamyl NH decreased slightly. Secondly, the temperature coefficient for the resonance signal of $Gly_I$-NH in the presence of guanidine hydrochloride is small below 60 °C and then becomes larger at higher temperatures. This phenomenon can be explained as follows: $Gly_I$-NH becomes more associated with solvent or with anion as a result of guanidine hydrochloride induced conformational change. Since such interactions become weakened at higher temperatures, the resonance signal for the $Gly_I$-NH protons shows an upfield shift in the temperature rises. The temperature coefficient of the upfield shift should be larger at $R$ = 2 where the association of NH with solvent or with anion, that is, the conformational change, is more important. However, the contribution of the association with solvent or with anion should be important only above 60 °C. At lower temperatures than 60 °C the conformation is very sensitive to the temperature change and a downfield shift of the $Gly_I$-NH signal due to the decrease of magnetic anisotropic effect cancels the upfield shift described above.

Deslauriers, Walter, and Smith[36] investigated the conformational change of cyclic peptide hormone oxytocin using $^{13}C$ NMR spectroscopy, and explained the long-range, pH-dependent effects on the $^{13}C$ NMR spectra in terms of the "through-space" mechanism. The argument here could be made firm by measurement of relaxation time ($T_1$) using pulse Fourier transform $^{13}C$ NMR spectroscopy.[37] If a large downfield shift is always observed for such internal peptide protons as $Gly_I$-NH of cyclo-($Gly_I$-X-$Gly_{II}$)$_2$ on the addition of guanidine hydrochloride to a $Me_2SO$ solution of the cyclic peptide, the downfield shift can be used as a criterion for the peptide proton being internal.

In water different behavior was observed. The NH resonances of IV in water gradually shifted to higher field on gradual addition of guanidine hydrochloride or lithium bromide, the shift change of $Gly_I$-NH signal with the addition of guanidine hydrochloride being an exception (Table II). In

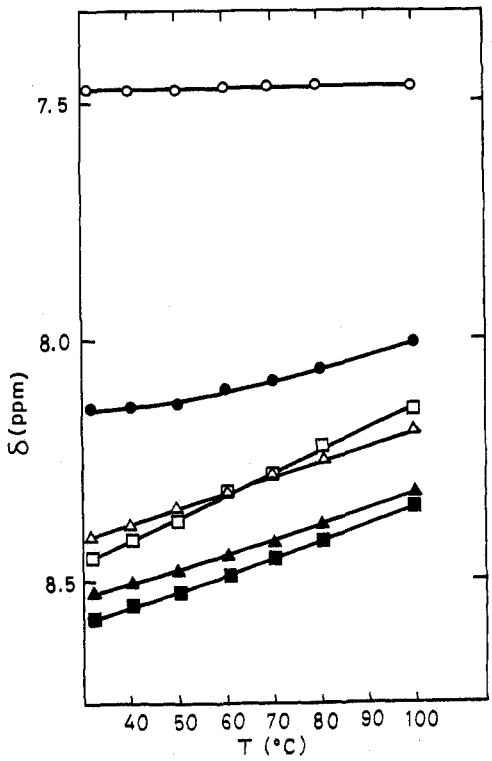


**Figure 3.** The temperature dependence of NH resonance signals of IV in $Me_2SO$-$d_6$: ○,● $Gly_I$-NH; △,▲ $Gly_{II}$-NH; □,■ Glu(OBzl)-NH. Full symbols represent the NH resonance signals in the presence of guanidine hydrochloride ($R$ = 2).

Table II only the chemical shift changes induced by the salts at $R$ = 10 are shown, which are much less marked than those observed in $Me_2SO$. The small changes observed in water imply that the added salts were well solvated, so that they did not bring about a serious conformational change. In fact, no change of $J$ values was observed in water. The small changes also imply a strong solvation of the peptide protons by water. Similar shift changes have been observed for the poly(sodium glutamate)–LiBr–$H_2O$ system[38] in the same concentration range of LiBr as employed in the present experiments. In the poly(sodium glutamate) case, the changes have been interpreted in terms of the dehydration of the polymer by an electrostriction effect. The same explanation could be applied to the present case, because the upfield shift of the resonance signal of $Gly_{II}$-NH protons was far greater than that of $Gly_I$-NH protons.

**Acknowledgment.** The authors wish to thank Dr. I. Morishima and Mr. H. Masuda for 220-MHz NMR measurements and their helpful discussions.

**Registry No.**—I, 51782-82-2; II, 59092-61-4; III, 59092-62-5; IV, 59092-63-6; V, 59092-64-7; VI, 59092-65-8; VII, 47094-17-7; VIII, 59092-66-9; IX, 59092-67-0; X, 59092-68-1; XI, 59092-69-2; glycine,

56-40-6; Boc-Glu(OBzl)ONSu, 32886-40-1; *p*-nitrophenol, 100-02-7; HCl-Gly-Glu(OBzl)-Gly-ONp, 59092-70-5; Boc-Glu(OBzl)-Gly-Gly-ONp, 59092-71-6; Boc-Glu(OMe)-OH, 45214-91-3; glycylglycine, 556-50-3; Boc-Asp(OBzl)-ONSu, 13798-75-9; Boc-Gly-ONSu, 3392-07-2.

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# Synthesis of 4-Tetracyclo[5.2.1.$0^{2,6}$.$0^{3,8}$]decene (2,4-Ethenotricyclo[3.3.0.$0^{3,7}$]octane)[1]


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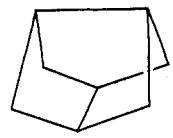
*Received February 17, 1976*


A short first synthesis of the cage olefin 2,4-ethenotricyclo[3.3.0.$0^{3,7}$]octane (**10**) is reported, from dihydrodicyclopentadiene **3** by way of a difunctionalizing and ring-inverting Wagner–Meerwein rearrangement, **4** → **5**, and a transannular carbenic insertion, **7** → **8** → **9**. The intramolecular reactions of the carbenes from *exo*-5,6-trimethylene-7-norbornanone tosylhydrazone (**14**) and the 2,3-olefinic analogue **24** have also been investigated, and are compared with the published reactions of the parent bicyclic carbenes from tosylhydrazones **17** and **26**, respectively.


The synthesis of cage-structured hydrocarbons and their derivatives has been of importance at several levels. The rigid and often symmetrical frameworks of such molecules have furthered understanding of the capabilities and limitations of diverse preparative methods, permitted the determination of new structure–reactivity relationships, and provided test compounds for force-field calculations of molecular energy and geometry. Examples of carbocyclic molecules in this class from which valuable information has been derived in recent years are adamantane,[2] bullvalene,[3] cubane,[4] iceane,[5] and twistane.[2a,c,6] Additional interest has been stimulated lately by the discovery of promising pharmacological properties of certain adamantane[7] and twistane[8] derivatives, apparently due to lipophilic character of the globular hydrocarbon moieties.

Another such spheroidal polycycloalkane, which has received relatively limited attention, is tricyclo[3.3.0.$0^{3,7}$]octane[9] (**1**). We report here a direct synthesis of 2,4-ethenotricyclo[3.3.0.$0^{3,7}$]octane (4-tetracyclo[5.2.1.$0^{2,6}$.$0^{3,8}$]decene) (**10**), the first 2,4-disubstituted derivative of this ring system and an olefin which should be useful for the construction of further new cage compounds. The molecule is the singly bridged relative of diethenotricyclooctane (**2**), recently prepared by Paquette and Wyvratt.[10]



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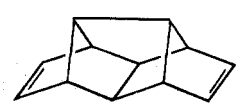

2

The central concepts in the synthesis of **10** were the endo → exo transformation of a 5,6-trimethylenenorborane skeleton by Wagner–Meerwein rearrangement,[11] **4** → **5**, and the transannular C–H insertion,[12] **8** → **9**, of a carbenoid constitutionally constrained against competing olefin formation by hydrogen shift.

## Results and Discussion

Reaction of excess cyclopentene with cyclopentadiene according to the procedure of Cristol and co-workers[13] gave rise to a 44% yield of *endo*-5,6-trimethylene-2-norbornene (**3**), which upon reaction with *m*-chloroperbenzoic acid provided the known epoxide[14] **4** in 93.5% yield. Treatment of the epoxide with anhydrous HBr in methylene chloride solution[14c] at 0 °C produced, after recrystallization, a 65% yield of *exo*-2-bromo-*exo*-5,6-trimethylene-*syn*-7-norbornanol (**5**), mp 92.0–93.2 °C, whose $^1$H NMR spectrum made clear that the desired Wagner–Meerwein rearrangement had occurred.