Synthesis and Conformation of 1,1,10,10-Tetramethyl[3.3]metacyclophane

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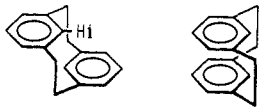
Abstract: Photoinduced $S_{RN}1$ reaction of 3-(*m*-bromophenyl)-3-methyl-2-butanone afforded 1,1,10,10-tetramethyl[3.3]-metacyclophane-2,11-dione (**8**) in 33% yield. Reduction of the carbonyl groups produced the desired hydrocarbon tetramethyl[3.3]metacyclophane **6**. The molecule of **6** adopts a syn chair–boat conformation in the crystalline state. The arene rings are tilted with respect to one another similar to the parent [3.3]metacyclophane. Two other conformers, syn chair–chair and syn boat–boat, are predicted to have similar steric energies to the one found in the crystalline state by molecular mechanics calculations. The variable-temperature ^1H NMR and ^{13}C NMR spectra supported the calculations. Three different ^1H NMR signals attributable to the same internal arene hydrogen can be observed at −96 °C, confirming the presence of the three syn conformers. Analysis of the temperature-dependent NMR spectra disclosed that the dynamic processes of **6** are the interconversion of these three syn conformers by "wobble" motions of the bridges and aromatic ring flips to give their respective mirror images. The barrier for the benzene ring flipping process was found to be smaller than those of the wobble motions of the 1,1-dimethylpropano bridge.

The stereochemical aspects of mobile cyclophanes have been of particular synthetic and theoretical interest for over the past two decades.[2] It has been generally known that [*m.m*]metacyclophanes have mainly two conformational versions, syn and anti. Only the anti isomers are known in [2.2]metacyclophane (**1**) and its derivatives, as long as they have at least one internal



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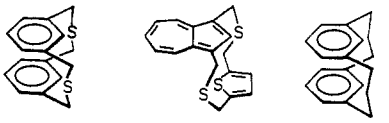
aryl hydrogen at C_8 or C_{16}.[3] Quite recently, however, two groups reported the synthesis of *syn*-[2.2]metacyclophane[4] (**2**) and its derivatives[5] and the facile conversion of these compounds to the anti forms at 0 °C by aromatic ring flipping. The energy difference ($\Delta H°$) between the syn and the anti conformers has been estimated to be ~17 kcal/mol,[4b,6] and the conformational equilibrium is completely in favor of the anti isomer.

The ^1H NMR chemical shift of the internal aryl hydrogens can be a useful probe for the assignment of structure, because the magnetic anisotropy due to the facing benzene ring causes the internal hydrogen (H_i) of **1** to exhibit an extensive up-field shift (δ 4.17). On the other hand, the corresponding hydrogen of **2** stays in the usual aromatic chemical shift region (δ 6.58).

By analogy with the [2.2]metacyclophanes, it was thought that even in larger metacyclophanes the anti conformation should be predominate over the syn form. However, contrary to the general belief, Mitchell and co-workers demonstrated that the predominant conformation of 2,11-dithia[3.3]metacyclophane (**3**) is syn in both the solid state and in solution.[7] Since then, the syn conformations



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have been confirmed in almost all conformationally mobile dithia[3.3]metacyclophanes.[8]

Unfavorable torsional strain (Pitzer strain) within the 2-thiapropano chains of the anti isomer has been proposed to be responsible for the predominance of the syn isomer.[9] However, in a few cases that contain sterically bulky atoms on the aryl rings, the anti isomer is known to be favored despite the small activation barriers for flipping of both of the aromatic rings. 2,13-Dithia[3.3]azulenothiophenophane (**4**) is such a case,[10] and the equilibrium constant between syn and anti isomers changes signifcantly with the temperature.

The syn isomer is also favored with [3.3]metacyclophane (**5**) itself. The synthesis of this interesting compound has been carried out by several groups. Misumi[11a,b] and Vögtle[11c] independently reported the synthesis by the thermal SO_2 extrusion method from the disulfones obtained by the oxidation of dithia[4.4]metacyclophanes. Inazu's method was the double alkylation of *m*-bis(halomethyl)benzenes.[11d,e] A ring-expansion approach from small congeners has also been used.[11f] Semmelhack[11g] employed a unique double nucleophilic aromatic substitution onto the chromium tricarbonyl complexed benzene ring.

Considerable effort has been expended in the analysis of the dynamic processes that occur with mobile [3.3]metacyclophanes.[8,12] Primarily, two processes have been deduced from

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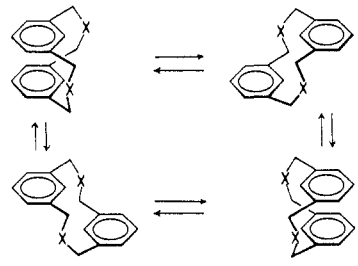


Figure 1. Syn-syn, anti-anti, syn-anti interconversion of [3.3]metacyclophanes via benzene ring flipping.

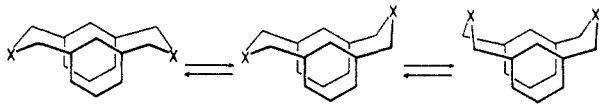


Figure 2. Interconversion between three syn forms of [3.3]metacyclophanes by wobble motion of the bridges.

variable-temperature NMR studies. One is the conformational change involving aromatic ring flipping such as syn–syn, syn–anti, and anti–anti interconversion (Figure 1). The other is a "wobble" motion of the bridging chains involving conformational isomerism between chair–chair, chair–boat, and boat–boat forms (Figure 2). Evidence for the former process has been obtained from the significant temperature dependence of the chemical shifts of the aromatic hydrogens in **4**.[10] The existence of the wobble motion can be seen visually in the crystalline state of 2,11-dithia-9,18-dimethyl[3.3]metacyclophane,[13] where both the syn chair–chair and the syn chair–boat forms coexist, though the latter is minor (20%).

Coalescence of the AB quartet of the benzylic CH_2 groups resulting in sharp singlets at higher temperature has been taken as evidence of a facile syn–syn interconversion via anti forms by aromatic ring flipping in **3**. It has been claimed that accidental chemical shift equivalence of the geminal benzylic hydrogens by the wobble motion of the bridge cannot be ruled out as an interpretation of this ^1H NMR behavior. Recently, Semmelhack and co-workers proposed that the wobble motion of the propano bridge in the parent [3.3]metacyclophane (**5**) is the main dynamic process and ruled out syn–syn interconversion.[14] This conclusion attracted our attention because we had previously demonstrated the existence of the aromatic ring flipping process with dithia[3.3]azulenophanes.[9,10,15]

In this article, we describe the novel synthesis of 1,1,10,10-tetramethyl[3.3]metacyclophane (**6**), the methyl substituents of which should be a good NMR probe for the dynamic processes, using the photoinduced $S_{RN}1$ reaction.[16] Evidence for syn–syn interconversion by aromatic ring flipping was obtained through variable-temperature ^1H and ^{13}C NMR studies. The unique chair–boat structure of the syn form was obtained by X-ray crystallographic analysis of **6**.

Results and Discussion

Synthesis of 6. As part of our synthetic program in the use of radical cyclizations for macrocycles containing aromatic rings, we have utilized a photoinduced double $S_{RN}1$ reaction.[17] Sterically

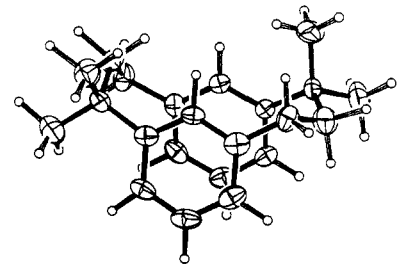


Figure 3. ORTEP drawing of **6**.

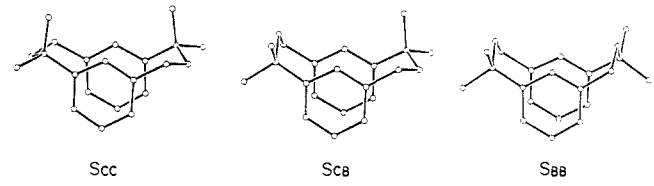
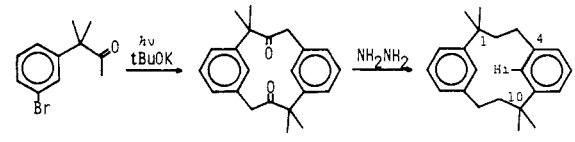


Figure 4. Three syn conformers of **6**.

congested carbocycles such as **6** are obvious current targets because the use of reactive σ-phenyl radicals can overcome the difficulties involved in ring closure.[18] Photoirradiation of 3-(*m*-bromophenyl)-3-methyl-2-butanone (**7**), which can be derived easily from



m-bromophenylacetonitrile, in the presence of excess KO^{t}Bu in liquid ammonia gave 1,1,10,10-tetramethyl[3.3]metacyclophane-2,9-dione (**8**) in 33% yield. Treatment of **8** with anhydrous hydrazine in diethylene glycol produced the desired hydrocarbon **6** in 84% yield.

X-ray Crystallography. The ORTEP drawing of **6** is shown in Figure 3. When compared to the ubiquitous syn chair–chair conformations of many [3.3]metacyclophanes, **6** has a rather unique syn chair–boat conformation in the solid state. A similar structure has been reported for 9-phenyl-2,11-dithia[3.3]metacyclophane in its crystalline state.[19] The two benzene rings of **6** are not parallel but are tilted by an angle of 21.8° so as to shift the two internal aryl carbons (C_9 and C_{18}) toward each other. Similar tilting of the two benzene rings (24°) has been observed in the parent compound **4**. Although a twist of the benzene rings by ca. 15° about an axis through the center of each ring has been reported in **4**, the two benzene rings of **6** are not twisted at all. The shortest transannular distance between the two arene carbons is 2.99 Å in **6**. It is shorter than the normal arene–arene stacking distance of 3.4 Å, suggesting some repulsion between the two π-electron systems. As a result of the transannular π–π repulsion, the two internal arene carbons bend away from each other. Hence, the two benzene rings deform into boat forms, although the outward displacement of these carbons from their respective best planes of the bottom part of the boat form is very small (0.048 Å, the average of the two). Carbon–carbon bond distances and angles agree well with normal values. The mean values are as follows: sp^2–sp^2 (benzene) 1.380 (12) Å, sp^2–sp^3 1.529 (12) Å, sp^3–sp^3 1.535 (14) Å; sp^2–sp^2–sp^2 119.4 (6)°, sp^2–sp^2–sp^3 120.6 (6)°, sp^2–sp^3–sp^3 111.9 (7)°, sp^3–sp^3–sp^3 110.6 (8)°.

Molecular Mechanics Calculations of 6. In order to obtain information about the structure and conformational energies of

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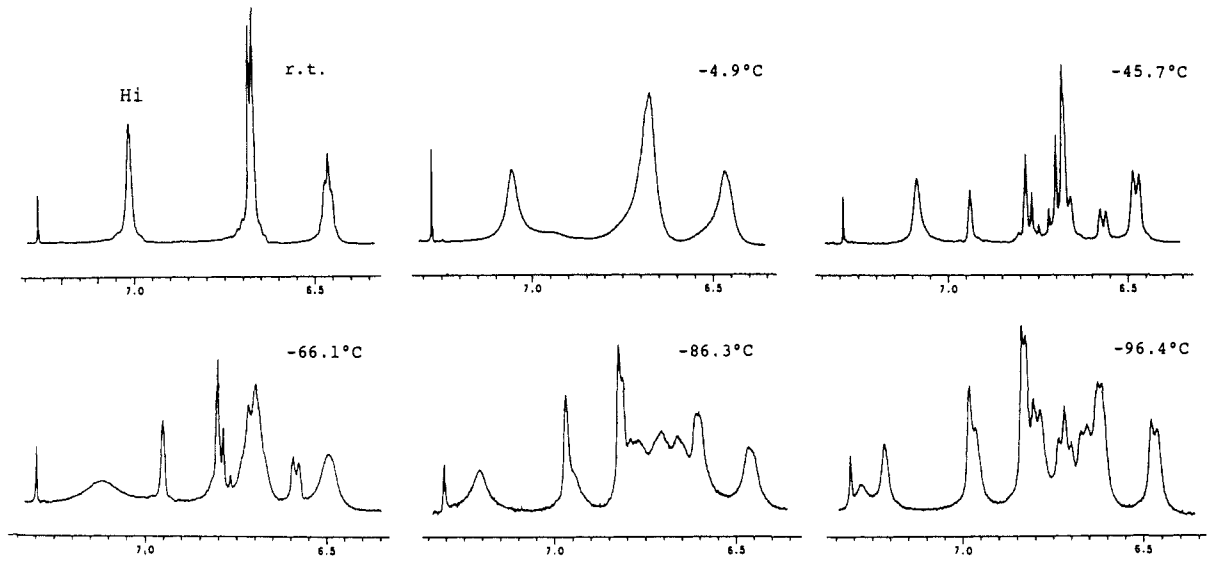

Figure 5. Aromatic region of variable-temperature ^{1}H NMR for **6**.

6 in solution, molecular mechanics calculations were performed. By use of the MM2[20] and our MMRS[21] programs, the latter of which generates all the plausible initial geometry for a molecule having a large-membered cyclic system, three syn (Figure 4) and several anti structures were obtained. To our surprise, and contrary to the general case, the syn boat–boat (S_{BB}) is the global energy minimum structure. However, the energy differences between this and the other syn structures are quite small [syn chair–chair (S_C) 0.02 kcal/mol, syn chair–boat (S_{CB}) 0.22 kcal/mol]. Thus, the introduction of methyl substituents has changed the stable structure of the parent [3.3]metacyclophane from the syn chair–chair to syn boat–boat. A similar structure has been reported in 1,3,10,12-tetrathia[3.3](2,6)pyridinophane in the crystalline state.[22]

Although the calculated global energy minimum structure is not the same one found in the crystal, it is not unnatural because packing energy in the crystal may affect the structure when we consider the calculated energy difference is so small. In a flexible molecule such as a macrocyclic compound, the structure obtained in the crystalline state is often not the true global energy minimum. In fact, 6,15-dimethyl-2,11-dithia[3.3]metacyclophane[23] is such a case. It is anti in the crystalline state,[24] whereas it is syn in solution.[8] The calculations also predict that the most stable structure of the anti forms has an additional 6.69 kcal/mol in steric energy relative to S_{BB}. This suggests a negligible population of the anti structure in the conformational equilibrium in **6**. A detailed analysis of the steric energies suggests that more than half of the extra energy of the anti conformation comes from torsional strain within the bridging chains, supporting our earlier rationale[9] for the predominance of the syn structure.

Dynamic NMR Spectroscopy. In accord with the molecular mechanics calculations, the predominance of the syn structure was suggested by comparison of ^{1}H NMR chemical shifts of the aromatic hydrogens with those of a reference compound (*m*-xylene) at room temperature. A small upfield shift (0.5–0.6 ppm) of the outer aromatic hydrogens together with the almost invariance of the internal aryl proton is compatible with the reported features of the syn structure.[8] The methylene signals due to the bridging chain appear as a symmetrical AA′BB′ pattern.[25] The singlet nature of the methyl signal suggests that some conformational equilibrium process that equalizes the four different magnetic environments of the methyl groups at room temperature must be operative.

In order to analyze the dynamic process, a sample of **6** was examined by ^{1}H NMR at 400 MHz over the temperature range 25 to −96 °C. Upon lowering the temperature, changes appear in both aliphatic and aromatic proton regions. The change of the signal due to the internal aryl hydrogen (H_i) is especially characteristic (Figure 5). It broadens and then splits into two peaks of unequal intensity below −10 °C. While the minor signal (at higher field) remains sharp at all temperatures below −45 °C, the major signal (at lower field) broadens further and resolves into two signals of unequal intensities at around −90 °C. At −96 °C, three signals at δ 7.28, 7.21, and 6.98 can then be assigned to be due to the same internal hydrogen (H_i). Other aromatic signals behave quite similarly, but due to the extensive overlapping of the separated signals at −96 °C, it is difficult to analyze the detailed mode of signal separation. Low resolution, even at 400 MHz, and complicated spectral changes prevent us from analyzing the splitting pattern of the ethylene part of the 1,1-dimethylpropano bridges at the lowest temperature. The behavior of the methyl signal, however, is very informative (Figure 6). The sharp singlet at room temperature becomes broad as the temperature is lowered and resolves into three singlets (δ 1.54, 1.33, and 1.27) at −45.7 °C. While the two outside signals remain as sharp singlets, the major peak in the middle broadens further and eventually resolves to give three sharp peaks. Altogether, five peaks (δ 1.55, 1.39, 1.30, 1.28, and 1.17) can be assigned to the methyl signals at the lowest temperature.

Parallel with the temperature-dependent ^{1}H NMR behavior, the broadening and the splitting are observed for all the carbon signals in the ^{13}C NMR spectra of **6**. All six different arene signals, three of which are already broadened at room temperature, broaden further as the temperature is lowered. At −41 °C, almost all peaks are split into two peaks of unequal intensities. While each of the minor peaks remains sharp at all temperatures below −50 °C, these major peaks broaden further and resolve into two or three peaks, respectively, at around −80 °C. Extensive overlap of many signals prevented unambiguous assignment of each of the separated signals at the lowest temperature (−91 °C). However, four signals (δ 139.4, 139.2, 138.8, and 138.5) can be assigned to a single quaternary carbon, C_4, since they are free from overlap with the other aromatic carbon signals. Tentative assignments of the chemical shift of each arene carbon signal at 24.2, −30.4, and −91.2 °C are summarized in Table I.

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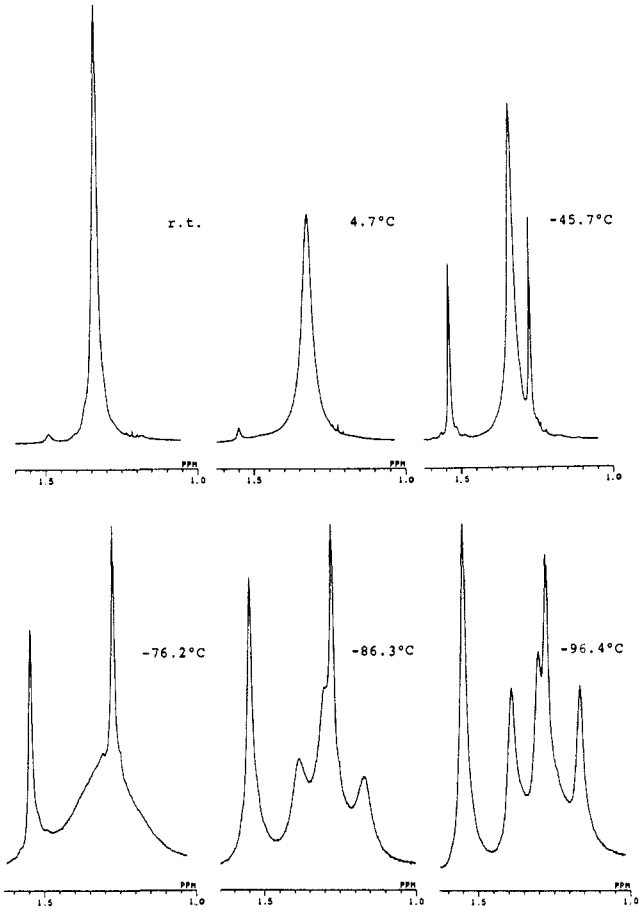


Figure 6. Methyl region of variable-temperature ^1H NMR for **6**.

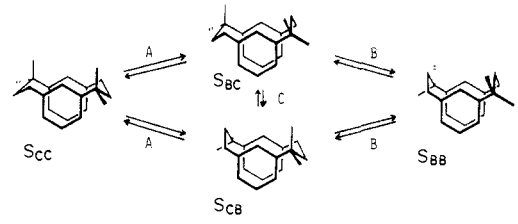
Table I. ^{13}C NMR Chemical Shifts for **6** at 24.2, −30.4, and −91.2 °C

	24.2	−30.4	−91.2
C_4	139.12	139.24	139.39 139.19
		138.90	138.78 138.51
$C_{5,7}$	126.31	126.35	127.03 126.76
		125.26	125.07 124.99
	123.10	123.70	123.88 123.56
		122.74	122.09 121.66
C_6	127.73	127.66	127.79 127.54
		127.48	127.41
C_8	146.46	146.34	146.39 146.21
C_9	129.90	130.83	131.53 130.66
		129.45	128.92

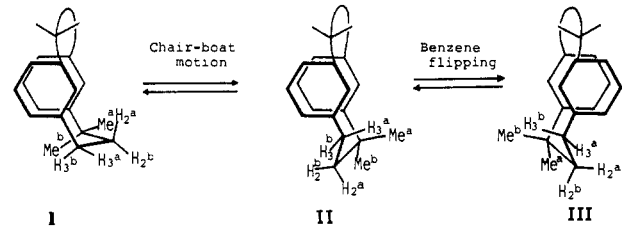
Dynamic Conformational Process of 6. The three low-field signals assignable to the internal aryl hydrogen (H_i) at −96 °C clearly prove the presence of the three different syn conformers. The ratio of the intensity of these signals (at δ 6.98, 7.21, and 7.28) is 45:34:21. The molecular mechanics calculations predict the ratio of the three syn conformers of S_{BB}, S_{CC}, and S_{BC} from their steric energies at −96 °C as 40:38:22. Unequivocal assignment of these individual signals cannot be given; however, there is good agreement between the calculated and observed ratios. The absence of any signal attributable to the H_i of the anti form at higher field indicates the negligible population of any one of the anti forms, even at the lowest temperature, supporting the results of the molecular mechanics calculations.

From the behavior of the temperature-dependent signal change of both H_i and the methyl group, the detail process of conformational interconversion can be clarified. Splitting of the H_i at higher temperature (−10 °C) suggests that one of the two processes A and B slowed down (Scheme I). Thus, if A is slowed down first, then process B slows and gives a second signal separation at −90 °C. Below this temperature, the interconversion rates between the three syn conformers are slow enough that the three

Scheme I



Scheme II



conformers each give their respective signals at different chemical shifts. From the signal separation pattern we can estimate the free energy of activation of these conformational interconversions. Values of ca. 13 and 9 kcal/mol have been obtained by the application of the coalescence temperature method[26] for the first and the second processes, respectively.

The splitting pattern of the methyl signals is compatible with these dynamic processes. If the A process slows down first the S_{CC} conformer, which gives two different signals because it has C_2 symmetry, freezes out. Slowing down the B process should give another two signals for the symmetrical S_{BB} and four signals due to S_{BC} (and/or S_{CB}). In theory, eight different signals should be observed at the lowest temperature. However, if process C, the synchronous in-and-out motion is still operative, the four different signals of S_{BC} (=S_{CB}) are reduced to two averaged signals. The six observed signals, two of which should be overlap at the lowest temperature, support our contention. In the ^{13}C NMR spectra, four different signals can be assigned to be due to the single aromatic carbon, suggesting the C process is also slowed down at the lowest temperature. The apparent inconsistency between the results of the ^1H NMR and ^{13}C NMR spectra studies can be explained if it is assumed that the larger chemical shift difference in the ^{13}C NMR allows the signal separation to be seen at higher temperature than in the ^1H NMR.

Since *pro-R* and *pro-S* methyl groups experience axial and equatorial positions equally during conformational interconversion, the temperature-dependent behavior of the methyl signals appears to be explicable without the help of benzene ring flipping. The chair–boat interconversion (I ⇄ II) scrambles axial and equatorial positions as suggested above and by previous authors.[14] However, this motion of the bridge alone cannot equalize their chemical shifts except by accidental coincidence (Scheme II). On the other hand, the aromatic ring flipping from II to its mirror image (III) interconverts the two geminal magnetically nonequivalent positions.

Evidence for aromatic ring flipping can be obtained by analysis of the signal pattern of the ethylene part within the 1,1-dimethylpropano bridges at room temperature. As noted before, they appear as a symmetrical AA'BB' pattern (Figure 7). Analysis of this pattern by computer simulation suggests that the chemical shifts of the two geminal hydrogens of each methylene group are identical with each other.[27] It is highly improbable

(26) Since it is known that the coalescence-temperature method cannot be applied correctly for two peaks of different intensities, a simple line-shape analysis method is also applied for the signal changes of the H_i using the CLATUX program (Binsh, G. *Top. Stereochem.* **1967**, *3*, 97) to give 13.4 (270 K) and 9.3 kcal/mol (190 K), respectively.

(27) Spectral simulation programs NMRIT/NMREN1 were used: *QCPE* **1968**, *No. 126/127*. Swalen, J. D.; Reilly, C. A. *J. Chem. Phys.* **1962**, *37*, 24.

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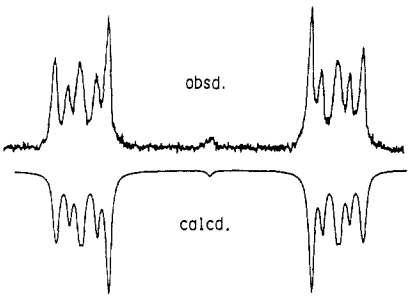
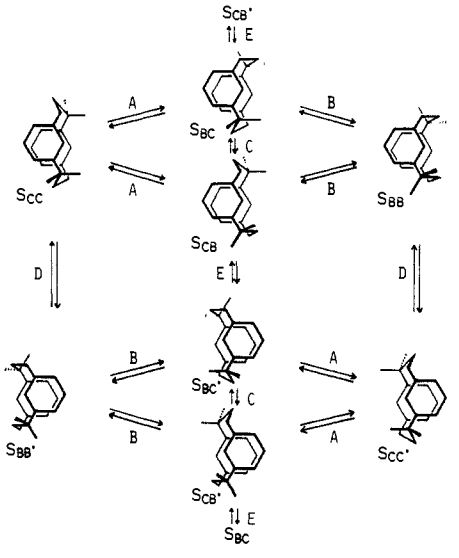


Figure 7. Observed and simulated spectra of ethylene signals of 1,1-dimethylpropano bridges. The following NMR data are obtained by application of the NMRIT/NMRENI programs: $\delta H_2^a = \delta H_2^b = 2.0722$ (3) ppm; $\delta H_3^a = \delta H_3^b = 2.7111$ (3) ppm; $J_{2a,2b} = -14.78$ (8) Hz; $J_{2a,3a} = J_{2b,3b} = 2.93$ (5) Hz; $J_{2a,3b} = J_{2b,3a} = 9.22$ (5) Hz; $J_{3a,3b} = -14.51$ (8) Hz.

Scheme III



that all three pairs of two magnetically nonequivalent hydrogens accidentally coincide at the same time by means of the wobble motion alone. However, ring flipping from II to III interconverts Me^a and Me^b as well as the two pairs of geminal hydrogens ($H_2^a \rightleftarrows H_2^b$, $H_3^a \rightleftarrows H_3^b$) (Scheme II). Only the ring flipping motion can equalize the magnetically nonequivalent hydrogens if it occurs rapidly, as was found to be the case from the above-mentioned chemical shift values at room temperature.

The dynamic processes of **6** can then be summarized as shown in Scheme III. Isolation of one symmetrical isomer (S_{CC} and S_{CC}') can be explained by freezing the two processes A and D if the two singlets of the methyl signals at δ 1.54 and 1.27 are due to the S_{CC} conformer. The major singlet at −46 °C must then be assigned to the methyl groups of the rapidly interconverting two conformers S_{CB} and S_{BB}. Complete coincidence of the two geminal methyls of S_{CB} and S_{BB} can be attained by the interconversions $S_{BB} \rightleftarrows S_{CB} \rightleftarrows S_{BC}' \rightleftarrows S_{BB}'$. Process B slows down next. The last motion, the ring-flipping process E is still operative at the lowest temperature on the ^1H NMR time scale, but slows down on the ^{13}C NMR time scale. The same explanation is valid if we assume that the first frozen isomer is S_{BB} and the $S_{CC} \rightleftarrows S_{CB} \rightleftarrows S_{BC}' \rightleftarrows S_{CC}'$ process is still operative. Two processes C and D, the latter of which is the direct interconversion between S_{CC} and S_{BB}', are not necessary for the interpretation of the temperature-dependent signal change.

Conclusion

The syn conformers of **6** were found to be predominate over the anti conformers at equilibrium, which is in accord with the results of the parent [3.3]metacyclophane. Thus, the introduction of the four methyl groups does not alter the stable conformer population. In this investigation we have identified the presence of a benzene ring flip process, which gives rise to syn–syn interconversion via undetectable anti isomers. The syn chair–boat (S_{CB}) conformer is the most favorable for the benzene ring flipping[15d] in **6**.

Experimental Section

General Procedures. ^1H NMR spectra were recorded on Hitachi R-22 (90-MHz) and JEOL-GX400 (400-MHz) spectrometers in $CDCl_3$ unless otherwise stated. Chemical shifts are reported in δ (ppm downfield from Me_4Si) using the following abbreviations: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. All melting points are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrometer. Column chromatography was performed using Merck silica gel (70–230 mesh).

3-(*m*-Bromophenyl)-3-methyl-2-butanone (7). To a solution of *m*-bromophenylacetonitrile (2.306 g, 11.8 mmol) in dry tetrahydrofuran (30 mL) was added potassium *tert*-butoxide (2.917 g, 26.0 mmol) at −40 °C, and the mixture was stirred for 8 min. After the addition of methyl iodide (1.95 mL, 29.1 mmol), the mixture was stirred for 1 h at room temperature, poured into dilute hydrochloric acid (10 mL), and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated to give 2-(*m*-bromophenyl)-2-methylpropionitrile (2.169 g, 82%) as a colorless oil which was used in the following reaction without purification: ^1H NMR (90 MHz, CCl_4) 1.70 (s, 6 H), 7.20–7.55 (m, 4 H).

To a solution of the above product (2.169 g, 9.68 mmol) in dry ether (30 mL) was added a solution of methyllithium in ether (0.8 M, 14.6 mmol) at 0–5 °C. After being stirred for 45 min at the same temperature, the mixture was acidified with hydrochloric acid. Stirring was continued overnight, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated. The residual oil was purified by column chromatography on silica gel (benzene eluent) to give **7** (2.051 g, 88%) as a colorless oil: ^1H NMR (90 MHz, CCl_4) 1.45 (s, 6 H), 1.87 (s, 3 H), 7.0–7.4 (m, 4 H); IR ($CHCl_3$) 2980, 1700, 1585, 1560, 1470, 1350, 1125, 1070 cm^{-1}. Anal. Calcd for $C_{11}H_{13}BrO$: C, 54.79; H, 5.43. Found: C, 54.84; H, 5.33.

1,1,10,10-Tetramethyl[3.3]metacyclophane-2,11-dione (8). A mixture of **7** (100 mg, 0.42 mmol), potassium *tert*-butoxide (280 mg, 2.50 mmol), and liquid ammonia (30 mL) was placed in a Pyrex dewar flask equipped with a dry ice condenser and irradiated with a high-pressure mercury lamp for 12 min. The solution was poured onto ammonium chloride, and the ammonia was allowed to evaporate. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated. Chromatography of the oily residue on silica gel (benzene eluent) afforded **8** (21.9 mg, 33%) as colorless prisms: mp 237–238 °C (from benzene); ^1H NMR (90 MHz) 1.42 (s, 12 H), 3.31 (s, 4 H), 5.82 (br s, 2 H), 7.24–7.62 (m, 6 H); IR (KBr) 2975, 1710, 1605, 1465, 1425, 1365, 1305, 1275, 1090, 1050, 1000, 910, 790, 720 cm^{-1}. Anal. Calcd for $C_{22}H_{24}O_2$: C, 82.46; H. 7.55. Found: C, 82.76; H, 7.37.

1,1,10,10-Tetramethyl[3.3]metacyclophane (6). Sodium (0.1 g) in dry diethylene glycol (10 mL) was heated under a nitrogen atmosphere until all the metal had disappeared. Anhydrous hydrazine (0.5 mL) and **8** (30.7 mg, 0.096 mmol) were added successively. The mixture was heated at 180 °C for 2 h and then at 210 °C for 6 h and cooled to room temperature. After addition of water, the mixture was extracted with ethyl acetate and the extract was washed with brine, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel to provide **6** as a colorless plates (23.6 mg, 84%): mp 87.5–88.5 °C (from ethanol); ^1H NMR (90 MHz) 1.36 (s, 12 H), 1.99–2.12 (m, 4 H), 2.63–2.87 (m, 4 H), 6.46–6.68 (m, 6 H), 7.00 (br s, 2 H); IR (KBr) 2950, 2900, 1610, 1585, 1490, 1440, 1385, 1365, 1180, 905, 840, 790, 770, 700 cm^{-1}. Anal. Calcd for $C_{22}H_{28}$: C, 90.36; H, 9.64. Found: C, 90.10; H, 9.81.

Single-Crystal X-ray Structure Determination of 6. The crystal data for **6** are as follows: orthorhombic; space group *Pcab*; $a = 18.379$ (5), $b = 18.840$ (9), $c = 10.149$ (4) Å; $V = 3514$ Å^3; $Z = 8$; empirical formula $C_{22}H_{28}$; molecular weight 292.44; D_{calcd} 1.106 g/cm^3, D_{obsd} 1.085 g/cm^3 by floatation in an aqueous KI solution. The three-dimensional X-ray data were collected by the use of graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å) on a Syntex R3 automatic four-circle diffractometer up to a maximum 2θ of 55.0°. The intensity data of 4512 independent reflections were collected and 1376 with $|F_o| > 4.5\sigma|F_o|$ were used in the present X-ray analysis. The structure was solved by the direct method (MULTAN78). All non-hydrogen atoms were located on the initial *E* synthesis. Hydrogen atoms were included in the calculated positions (C–H 1.08 Å). Block-diagonal least-squares refinements with anisotropic 22 non-hydrogen atoms and 28 isotropic hydrogens have converged to a conventional *R* factor of 0.092. All the calculation were done on a HITAC M-200H computer of the Hiroshima University using a struc-

ture analysis program system UNICS3.[27] Further results of the crystallographic experiment are available and are described in the supplemental material paragraph.

Molecular Mechanics Calculation. All the plausible initial geometries of **6** were generated automatically with use of our program (MMRS) using 30° of dihedral angle resolution. The closure bond was chosen to be the bond between C_2 and C_3. The constraints of the closure bond distance and angle were chosen to be in the range of 1.0–2.3 Å and 90–130°, respectively. The resulting 28 geometries that satisfy the ring closure criteria were then minimized with Allinger's MM2 force field using the following optional parameters for the benzene sp^2 carbon:

torsion	V1	V2	V3
1-2-2-2	-0.270	9.950	0.0
1-2-2-5	0.0	9.950	0.0
2-2-2-2	-0.930	9.950	0.0
2-2-2-5	0.0	9.950	-1.06

stretching (2-2) 8.07 (ks) 1.3937 Å (Lo)

Ten initial geometries out of 28 initial sets converged to one of the three syn conformers. The steric energies of the three syn conformers are 9.26 (syn boat-boat), 9.28 (syn chair-chair), and 9.47 kcal/mol (syn chair-boat). The 18 remaining initial geometries gave several, so-called anti structures. The steric energy of the lowest one of these anti forms was 15.95 kcal/mol.

Variable-Temperature NMR Spectra. A sample of the hydrocarbon **6** was dissolved in a mixture of CS_2-CD_2Cl_2-$CDCl_3$ (1:1:1). Spectra (^1H) were recorded on a JEOL GX-400 and are reproduced in Figures 5 and 6. Variable-temperature ^{13}C NMR spectra (100 MHz) were measured in a mixture of CS_2-CD_2Cl_2 (2:3). A spectra width of 20000 Hz, a filter bandwidth of 10000 Hz, an acquisition time of 1.25 s, a pulse width of 4.5 s, and 16000 data points with 1.5-s pulse delay were used.

Registry No. 6, 116633-68-2; **7**, 109757-55-3; **8**, 109757-64-4; (*m*-bromophenyl)acetonitrile, 31938-07-5; 2-(*m*-bromophenyl)-2-methylpropionitrile, 90433-20-8.

Supplementary Material Available: Tables of the final atomic coordinates, isotropic and anisotropic thermal parameters, bond distances, and bond angles and variable-temperature ^{13}C NMR spectra for **6** (7 pages). Ordering information is given on any current masthead page.

Solid-Phase Synthesis of Porcine Cardiodilatin 88†

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Abstract: An 88 amino acid residue peptide, porcine cardiodilatin pCDD 88, first isolated from porcine atria by a brain–gut hormone isolation protocol, was synthesized on an improved solid-phase peptide synthesizer and then purified. The homogeneity and primary structure were confirmed by several criteria, including two types of immunoassay. The synthetic material confirmed the proposed primary structure of natural pCDD 88. The results also illustrate the utility of solid-phase automated peptide synthesis using the t-Boc strategy in combination with HF cleavage for the preparation of relatively large peptides (M_r ca. 9400).

By functional and morphological methods it was revealed that mammalian atrial myocytes contain biologically active peptides stored in specific secretory granules.[1] Recently, several structurally related peptides have been isolated from mammalian atria and characterized. At the same time, the precursor of the atrial peptide of several species was deduced from cDNA analysis.[2] These peptides were termed collectively atrial natriuretic factor (ANF) or atrial natriuretic peptide (ANP), which have potent natriuretic, diuretic, and vasorelaxant activities. There is now agreement that atrial myocyte granules contain the pre-hormone consisting of 126 amino acid residues and that it's C-terminal fragment is a circulating form.[2] However, very little is known about the precursor itself as well as the biosynthetic pathways to these molecules.

Cardiodilatins (CDDs) were first isolated from porcine atria and characterized as atrial peptides by Forssmann et al.[3,4] The amino acid sequence of pCDD is similar to that of human τ-ANP.[5] The primary structures of porcine and human pro-atrial peptides are shown in Figure 1. Besides pCDD 126, pCDD 88 was also isolated from porcine atria in significant amounts[4] according to the isolation strategy for gastrointestinal hormones.[6] In this method, peptides in crude extracts were adsorbed on alginic acid and ethanol precipitation was employed as further purification. The primary structure of pCDD 88 is identical with the positions 39–126 of pCDD 126 (Figure 1), and it is believed[7] that pCDD 88 is generated intact in the biosynthetic pathways. On the other hand, immunoassays in combination with high-performance liquid chromatography (HPLC) showed that pCDD 88 might be an artifact of the isolation and purification procedure.[8]

Biologically active peptides are now possible to synthesize with an improved solid-phase procedure using high-quality reagents. The purpose of this study is to test the feasibility of synthesizing an 88 amino acid residue peptide by using an automated syn-

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