Conformations of 24-Membered Ring Pseudopeptides Containing N,N'-Ethylene-Bridged Dipeptides Constructed from (S)-Alanine, -Leucine, -Isoleucine, and -Phenylalanine

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ABSTRACT: 24-Membered ring pseudopeptides, cyclo[Gly-eXX-Gly]$_2$ {eXX; N,N'-ethylene-bridged dipeptide, X=(S)-alanine(A), -leucine(L), -isoleucine(I), and -phenylalanine(F)} and cyclo[Sar-eXX-Gly]$_2$ {X = (S)-A and -F} were prepared and their conformations were investigated by ^{1}H and ^{13}C NMR spectroscopy in dimethylsulfoxide-d_6. It was found from NMR measurements that the major conformers ($\geq 90\%$ abundance) of the four cyclo[Gly-eXX-Gly]$_2$ and major ones ($\simeq 70\%$) of the two cyclo[Sar-eXX-Gly]$_2$ have C$_2$-symmetric structures, in which all peptide bonds of cyclo[Gly-eXX- Gly]$_2$ (X=A, L, and I) and cyclo[Sar-eAA-Gly]$_2$ are *trans*, while two Gly-eFF peptide bonds of cyclo[Gly-eFF-Gly]$_2$ and two Sar-eFF ones of cyclo[Sar-eFF-Gly]$_2$ are *cis* though the other peptide bonds are *trans*. ^{13}C spin-lattice relaxation times (T_1) of all macrocycles showed small contribution of segmental mobility to T_1. The structures of cyclo[Gly-eAA-Gly]$_2$ and cyclo[Sar-eAA-Gly]$_2$ were optimized by molecular mechanics on the basis of NMR data.
KEY WORDS Alanine / Cyclic Pseudopeptide / Isoleucine / Leucine / Molecular Mechanics / NMR Spectra / Phenylalanine / Relaxation Time / Piperazin-2-one / Conformation /

Recently, many workers[1] have studied the preparations, functionalities[2] and biological activities[3] of various types of pseudopeptides which are compared with naturally occurring peptides. In these investigations, structural studies of pseudopeptides are important for examining the functionalities and biological activity/structure relationships.[4] Goodman *et al.*[5] synthesized 14-membered cyclic opioid pseudopeptides which are conformationally constrained analogs of the linear native dermorphin, and studied their preferred conformations experimentally and theoretically.

The authors also prepared various pseudopeptides containing lipophilic and conformationally restricted N,N'-ethylene-bridged dipeptides (eXX), to examine the fuctionali-

ties[6-8] and biological activities.[9] These conformationally restricted macrocycles are useful for the inclusion of inorganic[6] and organic[7,8] substrates in organic solvents such as chloroform, acetonitrile, etc., because of the lipophilicity of the macrocycles. Therefore, the conformations of a series of macrocycles must be examined in more detail in order to understand their functionalities more precisely. The authors have already examined the conformation[10] of a 24-membered ring pseudopeptide, cyclo[Gly-eVV-Gly]$_2$ {V= (S)-valine}.

In this paper, the conformations of other 24-membered ring analogs, cyclo[Gly-eAA-Gly]$_2$ (1), cyclo[Sar-eAA-Gly]$_2$ (1S), cyclo[Gly-eLL-Gly]$_2$ (2), cyclo[Gly-eII-Gly]$_2$

(3), cyclo[Gly-eFF-Gly]$_2$ (4) and cyclo[Sar-eFF-Gly]$_2$ (4S) were investigated by ^{1}H NMR measurements in dimethylsulfoxide (DMSO)-d_6. Moreover, the ^{13}C relaxation times (T_1) were measured.

The conformations of 1 and 1S were optimized by molecular mechanics (MM2)[11] based on ^{1}H NMR data.

EXPERIMENTAL

Preparation of Six Macrocycles and N,N'-Ethylene-Bridged Dipeptides (eXX-OMe) as Their Units

N,N'-Ethylene-bridged dipeptides, eAA-OMe,[12] eLL-OMe[7] and eFF-OMe[13] and a macrocycle, 2,[7] were prepared by our previous method.

Methyl (2S,3'S)-3-methyl-(2'-oxo-3'-*sec*-butyl-1'-piperazinyl)pentanoate (eII-OMe) was prepared according to a method similar to that used for the preparations of the other dipeptides. However, the esterification (20 days) of N,N'-ethylene-bridged bis-(S)-isoleucine{(2S,7S)-2,7-di-*sec*-butyl-3,6-diazaoctanedioic acid} by the thionyl chloride/methanol method and successive cyclization (50 days) in boiling xylene proceeded very slowly because of steric hindrance of *sec*-butyl group of isoleucine. Fortunately, eII-OMe thus obtained as an oily material in a 55% overall yield proved to be almost pure by NMR, IR, MS, and TLC. To obtain more precise physical and analytical data, eII-OCH$_3$ was coupled with *t*-butoxycarbonyl-glycine (Boc-Gly-OH) by the DCC (dicyclohexylcarbodiimide) method, and successively hydrolyzed in a MeOH–H$_2$O (1:1) solution of NaOH. Boc-Gly-eII-OH (5) obtained as a powder was purified by silica gel column chromatography and used as the unit of 3.

5: Yield 52%; mp 65—70°C; [α]$_D$= −32.9 deg dm^{-1} g^{-1} cm^3 (in methanol), MS: m/z 427 (M$^+$). IR (Nujol): 1735 and 1655 cm^{-1}. ^{1}H NMR: δ in ppm from TMS (CDCl$_3$): 7.8—6.7 (br s, 1H, COOH); 5.75 (s, 1H, NH);

5.00 (d, J=10.4 Hz, 1H, CH); 4.86 (d, J=7.9 Hz, 1H, CH); 4.07 (s, 2H, CH$_2$); 3.76—3.30 (m, 4H, CH$_2$—CH$_2$); 2.06 (br s, 1H, CH); 1.87 (br s, 1H, CH); 1.53 (m, 1H, 1/2CH$_2$); 1.44 (s, 9H, 3CH$_3$); 1.4 (overlapped signal, 1H, 1/2CH$_2$); 1.16 (m, 2H, CH$_2$); 1.03 (d, J=6.7 Hz, 3H, CH$_3$); 1.01 (d, J=6.7 Hz, 3H, CH$_3$); 0.91 (t, J=7.3 Hz, 6H, 2CH$_3$). *Elemental analysis.* Found: C=58.48%; H=8.78%; N=9.73%. Calcd for C$_{21}$H$_{37}$N$_3$O$_6$·1/4H$_2$O: C=58.38%; H=8.75%; N=9.73%.

Macrocycles, 1, 1S, 3, 4, and 4S, were prepared by a method[7] similar to that reported earlier and recrystallized from methanol, ethyl acetate, acetonitrile, chloroform and methanol–chloroform (1:1), respectively. The cyclization yields calculated from the free carboxylic acids of Boc-octapeptides were 18, 49, 34, 70, and 34% for 1, 1S, 3, 4, and 4S, respectively.

1: mp 236—240°C; [α]$_D$= +28.0 deg dm^{-1} g^{-1} cm^3 (in EtOH); MS: m/z 564(M$^+$). *Elemental analysis.* Found: C=47.58%; H=6.63%; N=18.41%. Calcd for C$_{24}$H$_{36}$N$_8$O$_8$·9/4H$_2$O: C=47.36%; H=6.75%; N=18.52%.

1S: mp 205—209°C; [α]$_D$= −59.4 deg dm^{-1} g^{-1} cm^3 (in MeOH); MS: m/z 592 (M$^+$). *Elemental analysis.* Found: C=49.16%; H=7.16%; N=17.56%. Calcd for C$_{26}$H$_{40}$N$_8$O$_8$·5/2H$_2$O: C=48.97%; H=7.11%; N=17.57%.

3: mp >300°C; [α]$_D$= −58.9 deg dm^{-1} g^{-1} cm^3 (in MeOH); MS: m/z 732 (M$^+$). *Elemental analysis.* Found: C=57.67%; H=8.32%; N=14.88%. Calcd for C$_{36}$H$_{60}$N$_8$O$_8$·H$_2$O: C=57.58%; H=8.32%; N=14.92%.

4: mp 192—200°C; [α]$_D$= +110 deg dm^{-1} g^{-1} cm^3 (in CHCl$_3$); MS: m/z 868 (M$^+$). *Elemental analysis.* Found: C=62.09%; H=5.83%. N=11.96%. Calcd for C$_{48}$H$_{52}$N$_8$O$_8$·1/2H$_2$O·1/2CHCl$_3$: C=62.12%; H=5.75%; N=11.95%.

4S: mp 177—181°C; [α]$_D$= −108 deg dm^{-1} g^{-1} cm^3 (in CHCl$_3$); MS: m/z 896 (M$^+$). *Elemental analysis.* Found: C=62.00%; H=6.66%; N=11.54%. Calcd for C$_{50}$H$_{56}$N$_8$O$_8$·4H$_2$O: C=61.97%; H=6.66%; N=11.56%.

Measurements

JEOL GX-400 (NMR spectra), JASCO DIP-320 (optical rotation at room temperature), and JASCO IRA-1 (IR spectra) were used for the measurements of all samples. The macrocyclic peptide concentrations in ^{1}H and ^{13}C NMR measurements were 0.075 and 0.040 mol dm^{-3}, respectively, in DMSO-d_6. The chemical shifts were obtained in ppm relative to the signals of DMSO-d_6 (2.49 and 39.5 ppm for ^{1}H and ^{13}C spectra, respectively).

All signals were assigned by two-dimensional measurements. ^{13}C Spin-lattice relaxation times (T_1) were measured at 40°C using a 180°-τ-90°-AQ pulse sequence. Interval between pulse sequences was 10s.

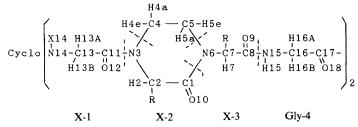## Molecular Mechanics Calculations (MM2)[11]

The molecular structures of **1** and **1S** deduced from ^{1}H NMR data were optimized by MM2, using the parameters (PEPCON) of Wolfe et al.[14]

Table I. ^{1}H NMR Chemical shifts and coupling constants for Gly and Sar residue protons of the major conformers of **1**,[a] **1S**,[a] **2**,[a] **3**,[a] **4**,[a] and **4S**[a] in DMSO-d_6 at 40°C

Macro-cycle	Chemical shift, δ/ppm (Coupling constants, J/Hz)					
	X14[b]	H13A and B		H15	H16A and B	
1	7.89	4.28	3.75	8.01	4.05	3.39
	(dd, 6.1, 4.9)	(dd, 17.1, 6.7)	(dd, 17.0, 4.3)	(dd, 7.9, 4.3)	(dd, 16.5, 7.9)	(dd, 15.6, 4.0)
1S	2.97	4.91	3.57	7.93	4.36	3.50
	(s)	(d, 16.5)	(d, 16.5)	(dd, 7.9, 3.7)	(dd, 17.1, 7.9)	(dd, 17.1, 3.7)
2	7.89	4.29	3.82	7.96	4.00	3.38
	(dd, 6.1, 4.3)	(dd, 17.1, 6.7)	(dd, 17.4, 4.0)	(dd, 7.6, 4.6)	(dd, 16.2, 7.6)	(dd, 15.9, 4.3)
3	7.82	4.48	3.71	8.07	3.95	3.33
	(dd, 7.0, 3.4)	(dd, 17.1, 7.3)	(dd, 17.4, 3.1)	(t, 6.1)	(dd, 16.5, 7.3)	(dd, 15.5, 4.9)
4	7.81	4.26	3.62	8.04	4.00	3.33
	(dd, 6.1, 4.3)	(dd, 17.1, 6.7)	(dd, 17.1, 3.7)	(dd, 7.6, 4.6)	(dd, 16.5, 7.9)	(dd, 16.5, 4.3)
4S	2.69	4.85	3.36	7.91	4.26	3.40
	(s)	(d, 16.5)	(d, 16.5)	(dd, 7.9, 4.3)	(dd, 16.5, 7.9)	(dd, 16.5, 4.3)

[a] Numbering of **1**, **1S**, **2**, **3**, **4**, and **4S**

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                              H4a
                               |
                         H4e-C4 ── C5-H5e
           X14 H13A         |  ><   H5a    R  O9      H16A
Cyclo      N14-C13-C11-N3        >|<     N6-C7-C8-N15-C16-C17-
           /   ||   O12'   \         >|<   H7   'H15 H16B  O18
           H13B                    H2-C2 ── C1
                                     |      \\
                                     R      O10
              X-1       X-2       X-3      Gly-4
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[b] X14 = H for **1**, **2**, **3**, and **4**, and X14 = CH$_3$ for **1S** and **4S**.

RESULTS AND DISCUSSION

Conformations of Macrocycles

It was found from ^{1}H NMR measurements that four cyclo[Gly-eXX-Gly]$_2$ (**1**, **2**, **3**, and **4**) used here consist of two conformers (major and minor), respectively. Also, the two cyclo-[Sar-eXX-Gly]$_2$ (**1S** and **4S**) all contain a major conformer, and show five minor amide proton signals reflecting the existence of other isomers.

Tables I and II show the ^{1}H NMR chemical shifts and coupling constants of the major conformers, which appear to have C_2-symmetric structures from their simple spectra. The assignments of their signals were made with NOE between the methine (H7) protons of X-3 and amide (H15) protons of Gly-4.

As shown in Table II, H2 signals of the major conformers are at 4.77, 4.67, 4.91, and 4.63 ppm for **1**, **1S**, **2**, and **3**. On the other hand, H2 signals of the minor conformers were observed at 4.36, 4.49, 4.20, and 4.0 ppm for **1**, **1S**, **2**, and **3**, respectively. Also, the chemical exchange signal between H2 proton of the major conformer and minor one was ascer-

Table II. ^{1}H NMR Chemical shifts and coupling constants for the ethylenic and the methine protons of eXX of the major conformers of **1**,[a] **1S**, **2**,[a] **3**,[a] **4**,[a] and **4S** in DMSO-d_6 at 40°C

Macro-cycle	Chemical shift, δ/ppm (Coupling constants, J/Hz)					
	H2[b]	Axial proton		Equatorial proton		H7
		H4a and H5a		H4e and H5e		
1	4.77 (q, 7.1)	3.4[c]	3.52 (ddd, 13.1, 9.8, 4.0)	3.87 (dt, 14.0, 3.7)	3.14 (dt, 12.2, 3.4)	5.09 (q, 6.9)
1S	4.67 (q, 7.3)	3.36 (ddd, 13.6, 10.3, 3.7)	3.64 (ddd, 12.7, 9.7, 3.5)	3.90 (bd, dt like 14.0)	3.14[c] (dt)	5.08 (q, 7.0)
2	4.91 (dd, 9.5, 4.6)	3.45[c]	3.51[c]	3.85[c] (dt)	3.18 (dt, 12.1, 4.3)	5.08 (dd, 8.9, 6.4)
3	4.63 (d, 11.0)	3.64—3.79[c]		3.43 (dt, 13.7, 3.8)	3.46 (dt, 15.0, 3.4)	4.61 (d, 9.2)
4	4.93 (t, 6.4)	2.96 (ddd, 12.8, 9.2, 3.7)	3.54 (ddd, 13.1, 8.6, 4.6)	3.75 (dt, 13.4, 4.3)	3.14[c] (dt)	5.37 (t, 7.9)
4S	4.83 (t, 6.4)	3.1[c]	3.68 (ddd, 12.8, 8.5, 4.1)	3.79 (dt, 13.4, 4.3)	3.2[c]	5.31 (t, 7.6)

[a] Refer to Table I for numbering of macrocycles.
[b] Peptide bonds between Gly-1 and eXX are *trans* for **1**, **1S**, **2**, and **3**, and *cis* for **4** and **4S**.
[c] Overlapping signals.

tained by the NOESY spectra. The relatively low field shifts of H2 protons of the major conformers of **1**, **1S**, **2**, and **3** are reasonably explained by the fact that the N3-C11=O12 peptide bonds are *trans*. In this case, H2 protons of these major conformers lie on the same plane of the N3–C11=O12 bonds, and are affected by the magnetic anisotropy of the amide groups (see structure I). Moreover, ^{1}H NMR data and CPK modelling suggest that all peptide bonds of these conformers are *trans*.

The N3–C11=O12 peptide bond of the major conformer of **4** (**4S**) is *cis* which was assigned by the observation of the NOE signal between the methylene (H13A and B) protons of Gly-1 (Sar-1) and the methine (H2) proton of X-2 though the other peptide bonds are *trans* (see structure II). The chemical shift values {4.93(4.83)ppm} of H2 of **4** (**4S**), for *cis* isomer are lower than the values {4.82(4.71— 4.79[15])ppm} for *trans* isomer because of the magnetic anisotropy of the phenyl group of Phe residues on the piperazine-2-one (MKP) ring.

Moreover, it is estimated from the integration of the amide (H15) proton signals and/or the α-methine (H2) ones that the ratios of *trans* amide bond (N3–C11=O12) conformers to *cis* ones are 90/10, 98/2, 99/1, and

10/90 for **1**, **2**, **3**, and **4**, respectively. The abundance of the major conformer and other total minor ones was estimated to be 68 and 32% for **1S** (72 and 28% for **4S**) from the integration of their NH proton signals.

As shown in Table II, the coupling constants (9.5 and 11.0 Hz for H2, and 8.9 and 9.2 Hz for H7, respectively) of **2** and **3** suggest that the α- and β-methine protons of **3**, and α-methin and either β-methylene ones of **2** in their X-2 and -3 are *trans* each other. The coupling constants (6.4 and 6.4 Hz for H2, and 7.9 and 7.6 Hz for H7, respectively) of **4** and **4S** show that the rotations about C2-R and C7-R (R = $CH_2C_6H_5$) axes are free or vibratile. It is suggested from the coupling constants of the ddd and dt signals for the axial (H4a and/or H5a) and the equatorial (H4e and/or H5e) protons that the MKP rings of all macrocycles exist in the medium between distorted and pseudochair forms.

Figure 1 shows the temperature dependence of amide proton (H14 and H15) signals. The temperature coefficients ($\times 10^{-3}$ ppm deg^{-1}) were -3.93, -6.25, -3.75, -3.25, -3.03, and -5.80 for the low field (H15) protons of **1**, **1S**, **2**, **3**, **4**, and **4S**, and -5.63, -5.88, -7.50, and
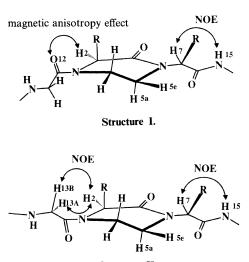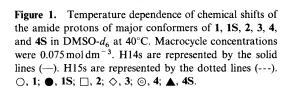
magnetic anisotropy effect

Structure I.

Structure II.

Figure 1. Temperature dependence of chemical shifts of the amide protons of major conformers of **1**, **1S**, **2**, **3**, **4**, and **4S** in DMSO-d_6 at 40°C. Macrocycle concentrations were 0.075 mol dm^{-3}. H14s are represented by the solid lines (—). H15s are represented by the dotted lines (---). ○, **1**; ●, **1S**; □, **2**; ◇, **3**; ◎, **4**; ▲, **4S**.

Table III. Torsional angles (°) calculated by MM2 based on the proposed conformations of **1** and **1S** in DMSO-d_6. The parenthesized parts are the torsional angles ($\pm 20°$) estimated from ¹H NMR data of Table I

| | 1 | | 1S | |
	Gly-1	Gly-4	Sar-1	Gly-4
ϕ	−143 (−135)	124 (130)	—	98 (120)
ψ	180 (160)	52 (30)	165 (155)	162 (165)

−5.75 for the high field (H14) ones of **1, 2, 3,** and **4**, respectively. These values suggest that both the former and latter have no intramolecular hydrogen bonds, referring to the values (−6 and −4.5) for NH of N-methylacetamide[16] and Gly₂ of cyclo[Gly₁-Pro-Gly₂-D-Ala-Pro][17] in DMSO-d_6. The absence of intramolecular hydrogen bond in **1, 1S, 2, 3, 4,** and **4S** is also supported by the values (−0.5 and 0.0×10^{-3}, respectively) for the amide protons of D-Ala and Gly-1 of
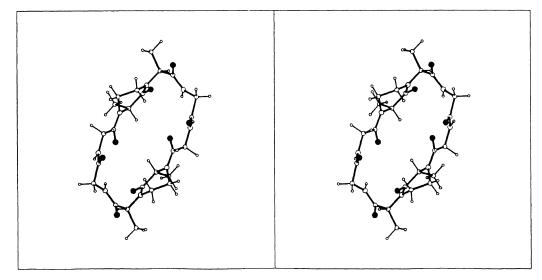

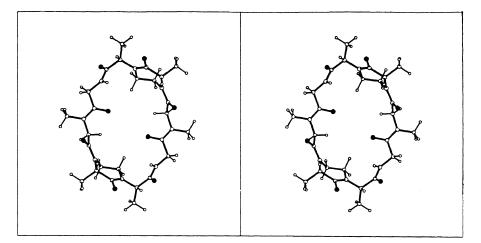**Figure 2.** Stereo-view of **1** optimized by MM2. ●, oxygen atoms.

Figure 3. Stereo-view of **1S** optimized by MM2. ●, oxygen atoms.

cyclo[Gly$_1$-Pro-Gly$_2$-D-Ala-Pro][17] with intramolecular hydrogen bonds. It is suggested from CPK modelling that 24-membered ring pseudopeptides, cyclo[Gly-eXX-Gly]$_2$ and cyclo[Sar-eXX-Gly]$_2$, have no intramolecular hydrogen bonds (O(10) or O(12)---H–N(15) for **1**, **2**, **3**, and **4**). The absence of intramolecular hydrogen bonds are also supported from the MM2 estimated with ^{1}H NMR data of cyclo[Gly-eVV-Gly]$_2$[10] in acetonitrile-d_3/dioxane-d_8 (v/v = 4/1) in addition of the ^{1}H and ^{13}C NMR and FTIR data of cyclo[Gly-eLL-Gly]$_2$[18] in chloroform-d_1.

On the basis of torsional angles (ϕ and ψ)[19] estimated[20] from the ^{1}H NMR data of Table I, the most reasonable calculated values of the torsional angles (ϕ and ψ) of **1** and **1S** are shown in Table III. The steric energy of **1** (**1S**) was obtained as 40.2 (50.7) kcal mol^{-1} by MM2. From the above results, the structure of **1** (**1S**) is proposed in Figure 2 (3). The skeleton structures of **2**, **3**, and **4** may be similar to that of **1** from the coupling constants of proton signals, though the peptide bonds of the Gly-eFF of **4** are *cis*. The skeleton structure of **4S** is similar to that of **1S** from the ^{1}H NMR

Table IV. ^{13}C NMR Chemical shifts of the skeleton (α- and ethylenic) and side chain β-carbons of all macrocycles,[a] and N–CH$_3$ carbons of **1S** and **4S** in DMSO-d_6 at 40°C

Macrocycle	α-Carbon				Ethylenic carbon		β-Carbon		
	C2	C7	C13	C16			C(X-2)	C(X-3)	N–CH$_3$
1	51.2	50.0	*[b]	41.6	40.3	38.4	12.8	16.3	
1S	51.1	50.1	48.3	*[b]	40.5	38.7	12.9	16.5	34.8
2	53.9	52.3	*[b]	41.7	*[b]	38.5	40.5	35.8	
3	57.8	60.7	*[b]	41.6	40.6	38.1	31.3	36.9	
4	56.5	55.3	*[b]	41.6	40.4	*[b]	36.7	33.1	
4S	56.1	55.5	48.1	*[b]	40.7	*[b]	36.6	33.2	34.2

[a] Refer to Table I for numbering of macrocycles, and β-carbons of side chains are C(X-2) and C(X-3) in X-2 and -3 residues, respectively.
[b] The methylene carbon signals overlap with those of DMSO-d_6.

Table V. Spin-lattice relaxation times NT_1(s)[a] of α-, β- and ethylenic carbons of **1**,[b] **1S**,[b] **2**,[b] **3**,[b] **4**,[b] and **4S**[b] in DMSO-d_6 at 40°C

Macrocycle (M_w)	Conc. mol dm^{-3}	α-Carbon				Ethylenic carbon		β-Carbon	
		C2	C7	C13	C16			C19	C20
1	0.075	0.32	0.32	*[c]	0.38	0.40	0.42	1.93	1.83
(565)	0.040	0.32	0.31	*[c]	0.33	0.39	0.42	1.84	1.83
1S (579)	0.075	0.31	0.33	0.32	*[c]	0.42	0.36	1.71	1.67
2	0.075	0.26	0.27	*[c]	0.30	0.40	*[c]	0.45	0.44
(733)	0.040	0.31	0.28	*[c]	0.33	0.47	*[c]	0.47	0.47
3	0.075	0.23	0.21	*[c]	0.25	0.25	0.28	0.22	0.26
(733)	0.040	0.26	0.25	*[c]	0.24	0.28	0.29	0.27	0.37
4	0.075	0.25	0.25	*[c]	0.28	*[c]	0.29	0.40	0.33
(869)	0.040	0.29	0.30	*[c]	0.26	*[c]	0.39	0.43	0.41
4S (883)	0.075	0.24	0.23	0.26	*[c]	*[c]	0.30	0.33	0.32

[a] Error range is ±0.02s.
[b] Refer to Table I for numbering of macrocycles.
[c] Overlapping signals.

data, though the peptide bonds of the Sar-eFF of **4S** are *cis*.

Table IV shows the ^{13}C NMR chemical shifts of the skeleton (α- and ethylenic) and side chain β-carbons of all macrocycles and *N*-methyl carbons of **1S** and **4S**.

^{13}C Spin-lattice Relaxation Time and Conformational Flexibility of Macrocycles

Table V shows the spin-lattice relaxation times (NT_1) of macrocycles (**1, 1S, 2, 3, 4**, and **4S**), where N represents the number of protons bound to a carbon. The relaxation times of the protonated backbone carbons (α-carbons and ethylenic carbons) of these macrocycles are measures of dynamic flexibility(mobility based on rotations around ϕ and ψ).[21,22] As discussed in the preceding section, all these macrocycles have C_2-symmetric structures, so that their molecular shapes should be similar in DMSO-d_6. From the molecular weight (∼800) and measured NT_1 values (0.2—0.5 s) of the α- and ethylenic carbons of these macrocycles, the correlation time (τ_C) is ∼10^{-10} s. In the case of extreme motion narrowing, the relation between T_1 and τ_C is approximated by eq 1[22]

$$\frac{1}{NT_1} \approx \frac{h^2 \gamma_C^2 \gamma_H^2 \tau_C}{r^6} \quad (1)$$

where γ_H and γ_C are the magnetogyric ratios of the proton and carbon nuclei, respectively. τ_C is determined by both overall rotational motion, τ_R, and intramolecular segmental motion, τ_g. τ_R is related to the radius (a) of the sphere and solution viscosity (η) by $\tau_R = 4\pi\eta a^3(3kT)^{-1}$. Assuming $\tau_C = \tau_R$ and proportionality between a^3 and the molecular weight, NT_1 is inversely proportional to the molecular weight.[22] Each τ_C for **1, 1S, 2, 3, 4**, and **4S** is determined mainly by τ_R, while the contribution of τ_g is negligible, because NT_1 of **1S, 2, 3, 4**, and **4S** are 0.95, 0.84, 0.66, 0.73, and 0.70 times as large as those of **1**. The molecular weights of **1S, 2, 3, 4**, and **4S** are 1.05, 1.30, 1.30, 1.54, and 1.59 times as large

as that of **1**, respectively. That is to say, the contribution of τ_g to τ_C can almost be neglected, though the small difference of NT_1 between **2** and **3** having the same molecular weight is considered due to difference in steric hindrance between *iso*- and *sec*-butyl groups. Therefore, fixations[1] of all macrocycles may be attributed to the *N,N'*-bridged ethylene groups in preference to the side chains of amino acid residues, and their flexibilities are similar. In these cases, macrocycle concentrations are disregarded, though NT_1 increases slightly with decrease of peptide concentration.[22]

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