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Conformational Study of a Tetraacyl Biosynthetic Precursor of Lipid A by NMR

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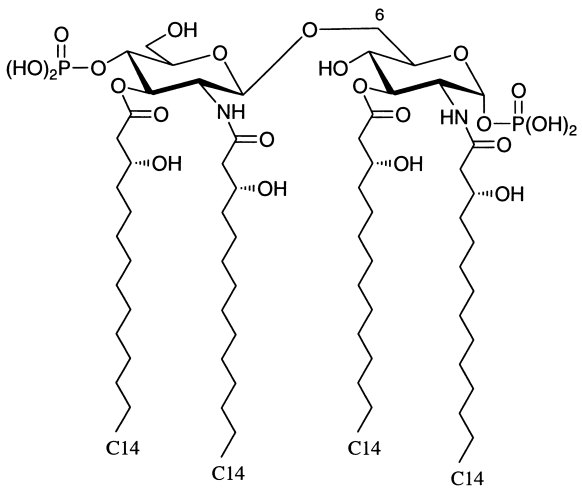
During the course of a conformational study of lipid A, which is a bioactive entity of lipopolysaccharide of the Gram-negative bacterial cell surface, the molecular conformation of its tetraacyl biosynthetic precursor in dimethyl sulfoxide was unambiguously determined by means of NMR using both 6-^{13}C-labeled and nonlabeled synthetic specimens. The conformation of the hydrophilic moiety was determined by an NMR analysis based on the spin-coupling constants and nuclear Overhauser enhancement data around the glycosidic linkage. The whole molecular shape of the glycolipid was then elaborated with the aid of molecular mechanics calculations.

The variety of important biological activities of the lipid A family, both toxic and beneficial,[1] to higher animals has been considered to be attributed to its diverse and specific mode of interactions between receptors on competent animal cells.[2,3] In order to elucidate the mechanism of the biological actions of lipid A analogues, it is therefore important to determine their conformations, particularly those in aqueous solutions and in bound states. As an initial step to this final goal, the conformation in an organic solvent was first studied, since no such precise analysis has yet been available, except for that of Wang and Hollingsworth.[4] Although they described the NMR spectra and the conformation of *Escherichia coli* lipid A, the study was performed in a mixed solution of pyridine-d_5, 37% deuterium chloride, methanol-d_4, and chloroform-d. The aim in their study was not to investigate a biologically important conformation, but just to obtain well-resolved NMR spectra of amphiphilic lipid A by using a designed solvent system which reduces the intermolecular hydrophilic interaction of the phosphorylated glucosamines and disperses the hydrophobic acyl groups.

The present paper deals with a conformational study of a synthetic pure specimen of a tetraacyl biosynthetic precursor **1** of lipid A (Chart 1) in dimethyl sulfoxide-d_6 (DMSO-d_6). Because the biosynthetic precursor shows antagonistic activity to abolish the endotoxic action of mature lipid A,[5] a comparative study on the conformation of endotoxic and antagonistic lipid A analogues is expected to be very important.

The conformation of a hydrophilic moiety composed of two glucosamine residues and two phosphate groups was first determined experimentally. To accomplish a quantitative conformational analysis, the use of spin-coupling constants ($^3J_{X,H}$), which are well known to depend on the dihedral angles of X–·–·–H, between nuclei X and proton, is generally desirable. The correctness of the conformational analysis based on $^nJ_{X,H}$ has been well proved, even for acyclic systems, as reported in a



1 : Biosynthetic precursor of lipid A
6-^{13}C-**1** (Carbon at 6-position is labeled with ^{13}C)

Chart 1.

study of a polyketide-derived natural product, okadaic acid.[6] This methodology was further applied to determine the configuration of maitotoxin and other natural products.[7] Though the $^nJ_{C,H}$ value has also played a key role in the conformational analysis of carbohydrates,[8] the low solubility and aggregation in several solvents has never allowed an NMR study of lipid A. For example, the maximum solubility of the biosynthetic precursor **1** in DMSO is ca. 1.5 mol dm^{-3}. At this concentration, a measurement using hetero half-filtered total correlation spectroscopy (HETLOC),[9] which has been successfully used in NMR studies of okadaic acid and maitotoxin[6,7] for $^nJ_{C,H}$ determination, failed to give a reliable result with the natural abundance of ^{13}C. The solubility problem could, however, be overcome by using a ^{13}C-labeled specimen.

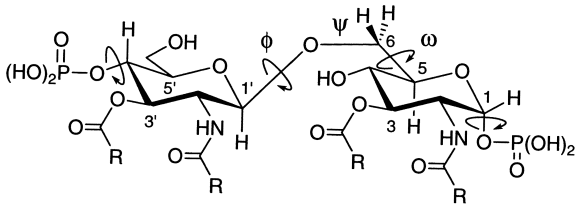


Fig. 1. Dihedral angles in the hydrophilic region of **1** to be determined by the use of both 6-^{13}C-labeled and nonlabeled specimens. The hydrophobic moieties are represented as R (RCO = (*R*)-3-hydroxytetradecanoyl).

The strategy for a conformational analysis of **1** in the present study was as follows (Fig. 1). The conformation of two phosphates was determined by an analysis of the Karplus equation with the $^3J_{P,H}$ values in NMR, and that of two glucosamine skeletons was easily clarified with the intraglycosidic $^3J_{H,H}$ values as well. For a quantitative discussion of the relative spatial arrangement of two glucosamines, information on the conformation of three bonds (C_5–C_6, C_6–O_6, and O_6–$C_{1'}$) is required.[10] This requirement is due to the well-known, conformationally less-restricted nature of this moiety of (1→6) disaccharides, as compared to other types of disaccharides, including (1→2), (1→3), and (1→4) ones, which have fewer bonds (two bonds) between the two pyranose rings, and are hence generally accepted to populate the close-to-parallel orientation at the transglycosidic C–H bonds.[11] In the present case (Fig. 1), the $^3J_{H,H}$ values of three well-resolved protons at C_5 and C_6 made it possible to determine the dihedral angle (ω) around the C_5–C_6 bond. The dihedral angle (ϕ) around the O_6–$C_{1'}$ bond was estimated based on the $^3J_{C6,H1'}$ value obtained from the NMR spectrum of a 6-^{13}C-labeled synthetic specimen.[12] For an unambiguous conformational discussion of the remaining C_6–O_6 bond, the combined consideration of NOE information around the glycosidic bond and an insight concerning the relative thermodynamic stability of rotamers were adopted because of a lack of the $^3J_{C1',H6}$ value. The quality of the analysis based on NMR was further polished for an estimation of the whole molecular shape with the aid of molecular mechanics calculations.

Results and Discussion

NMR Experiments. The syntheses of 6-^{13}C-**1**[12] and nonlabeled **1**[13] have already been reported. The NMR sample was prepared by dissolving 1.40 mg (1.0 μmol) of 6-^{13}C-**1** in 0.70 mL of DMSO-d_6. A sample of nonlabeled **1** was prepared in the same way, and gave the same NMR spectra as those of 6-^{13}C-**1**, except that protons at C_6 were split by $^1J_{C,H}$ (vide infra) in the latter. All spectra were collected at 30 °C with a JEOL JNM-LA500 or Varian UNITYplus600 spectrometer, and analyzed using the Felix® program (version 97.0, Molecular Simulations). Proton chemical shifts were referenced to residual protons of DMSO-d_6 (δ 2.565), and shown in ppm down field from the reference peak. Carbon chemical shifts were also referenced to the solvent (DMSO-d_6: δ 39.5).

Proton signals of 6-^{13}C-**1** in DMSO-d_6 were mainly assigned based on ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{1}H total correlation spectroscopy (TOCSY), and ^{1}H–^{1}H nuclear Overhauser enhancement spectroscopy (NOESY). The assignments have been summarized elsewhere,[12] and that of the protons on the glucosamine residue is indicated on a ^{1}H NMR spectrum in Fig. 2. The distinct signal of C_6 at δ 66.4 enabled us to easily assign H_4 and H_5 by a ^{13}C-^{1}H heteronuclear multiple-bond correlation (HMBC). The low-field protons at C_6 and $C_{6'}$ are designated with the character "a", and were assigned to be *pro-S* according to a literature reported by Meguro et al.[14]

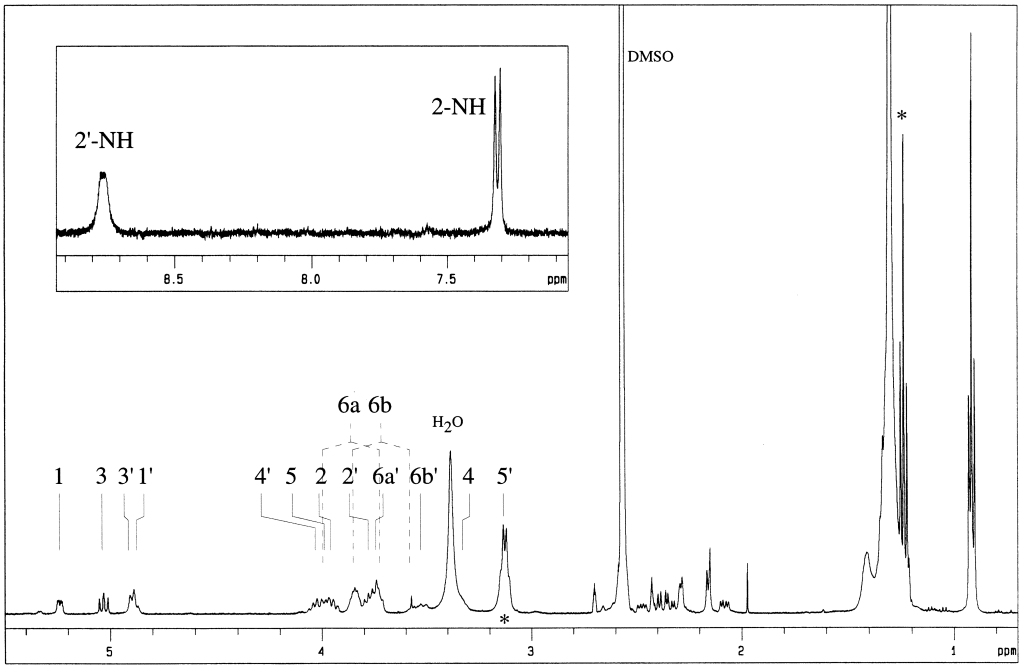


Fig. 2. A ^{1}H NMR spectrum (500 MHz) of 6-^{13}C-**1** in DMSO-d_6. Asterisks indicate the signals of triethylamine.

Table 1. Selected $^{n}J_{X,H}$ Values Important for the Conformational Analysis

Position	$^{n}J_{X,H}$/Hz[a)]
P_1/H_1	6.6[0.02]
$P_{4'}/H_{4'}$	6.4[0.02]
H_5/H_{6a}	2.7[0.1]
H_5/H_{6b}	6.3[0.1]
H_2/2-NH	9.0[0.02]
$H_{2'}$/2′-NH	6.0[0.02]
C_6/H_5	−2.3[0.8]
$C_6/H_{1'}$	4.6[0.8]

a) In brackets are given uncertainties (Hz) caused by digital resolutions of spectra.

The remaining *pro-R* protons on C_6 and $C_{6'}$, appearing at higher fields, are designated with the character "b".

Important $^{n}J_{X,H}$ values for the conformational analysis are summarized in Table 1. The values of $^{3}J_{P1,H1}$, $^{3}J_{P4',H4'}$, $^{3}J_{H5,H6a}$, $^{3}J_{H5,H6b}$, $^{3}J_{H2,2\text{-NH}}$, and $^{3}J_{H2',2'\text{-NH}}$ were successfully obtained from proton *J*-resolutional spectroscopy of nonlabeled **1**. The $^{2}J_{C6,H5}$ value was obtained from the splitting of the H_5/H_{6b} signal in *F*1 dimension, which appeared as an "exclusive COSY (E. COSY)"-like cross-peak structure[15,16] in a phase-sensitive ^{1}H–^{1}H NOESY spectrum (600 MHz) of 6-^{13}C-**1** obtained at 800 ms mixing time (Fig. 3). In a similar way, the $^{3}J_{C6,H1'}$ value was determined to be 4.6 Hz from the splitting of the $H_{6b}/H_{1'}$ signal in the spectrum (600 MHz) of phase-sensitive ^{1}H–^{1}H rotating frame nuclear Overhauser enhancement spectroscopy (ROESY) with a 150 ms mixing time (Fig. 4). In this way, by using the enriched compound, the ^{13}C–^{1}H spin-coupling constants ($^{n}J_{C,H}$), which have never been determined with a nonlabeled compound, as discussed above, were easily obtained. The digital resolutions in all cases are satisfactorily less than 0.8 Hz.

A phase-sensitive NOESY experiment (1000 ms mixing time) of nonlabeled **1** at 600 MHz (Fig. 5) shows correlations at $H_{1'}/H_{6a}$, $H_{1'}/H_{6b}$, (2-NH)/H_1, and (2-NH)/H_3.[10] Other NOESY build-up experiments with various mixing times (715–1105 ms) at 500 MHz gave similar spectra. By regarding a cross-peak of $H_{1'}/H_{3'}$ of diaxial relationship as a reference peak to be 2.65 Å, which is a calculated value on α-D-glucosamine by a semi-empirical molecular orbital calculation at the PM3 level of theory,[17,18] the distance between these hydrogen atoms could be estimated to be $H_{1'}/H_{6a}$ = 2.66 Å, $H_{1'}/H_{6b}$ = 2.69 Å, (2-NH)/H_1 = 2.85 Å, and (2-NH)/H_3 = 2.74 Å. These values were used only for the final evaluation of the computationally optimized conformations (vide infra).

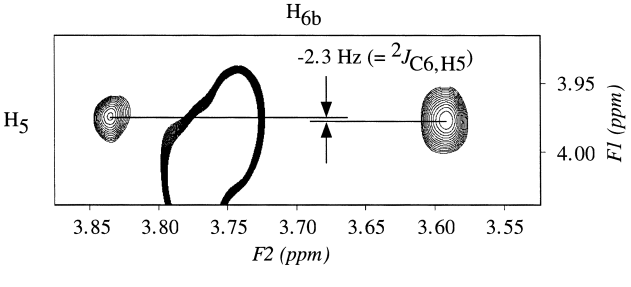


Fig. 3. A part of a phase-sensitive NOESY spectrum (600 MHz) of 6-^{13}C-**1** in DMSO-d_6. The mixing time was 800 ms, and the data size was $2K(t_2) \times 512(t_1)$ points for a spectral width of 6346 Hz. After carrying out the four-fold zero filling in *F*2, a skew squared sine-bell window function shifted by $-2\pi/5$ was applied in both dimensions prior to Fourier transformation. The correlation at H_5/H_{6b} appears like E.COSY cross-peak structure.

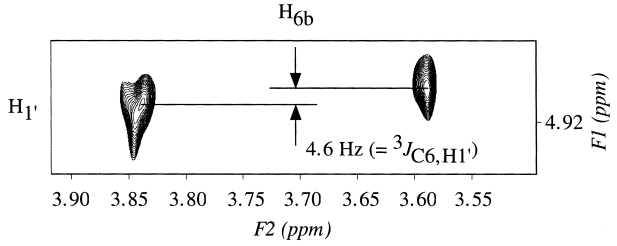


Fig. 4. A part of a phase-sensitive ROESY spectrum (600 MHz) of 6-^{13}C-**1** in DMSO-d_6. The mixing time was 150 ms. The data collection and the processing are same with that of NOESY (Fig. 3). The correlation at $H_{1'}/H_{6b}$ appears like E.COSY cross-peak structure.

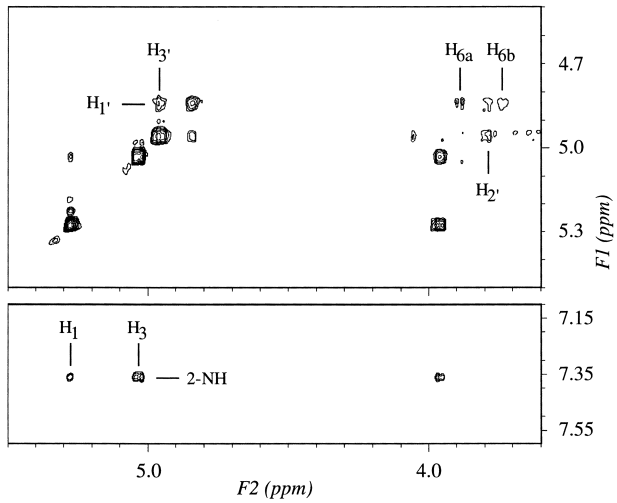


Fig. 5. A NOESY spectrum (600 MHz) of nonlabeled **1** in DMSO-d_6. The mixing time was 1000 ms.

Conformational Analysis. From intraglycosidic ^{1}H–^{1}H spin-coupling constants ($^{3}J_{H,H}$),[12] both of the glucosamine residues were found to occupy the normal $^{4}C_1$ chair conformation.[19] Therefore, the whole molecular shape of **1** depends upon the conformation of the C_5–C_6, C_6–O_6, and O_6–$C_{1'}$ bonds between two rings.

The conformational relationships of two oxygen atoms at C_5 and C_6 (dihedral angle (ω) of the O_5–C_5–C_6–O_6 bond) were determined from the $^{3}J_{H5,H6a}$, $^{3}J_{H5,H6b}$, and $^{2}J_{C6,H5}$ values listed in Table 1 as follows. According to Meguro's equations (Eqs. 1, 2, and 3) shown below,[14] the $^{3}J_{H5,H6a}$ (2.7 Hz) and $^{3}J_{H5,H6b}$ (6.3 Hz) values show that the ratio (Pg^-:Pg^+:Pt) of −*gauche* (gg), +*gauche* (gt), and *trans* (tg) conformers[20] is 0.46:0.47:0.07 (Fig. 6).

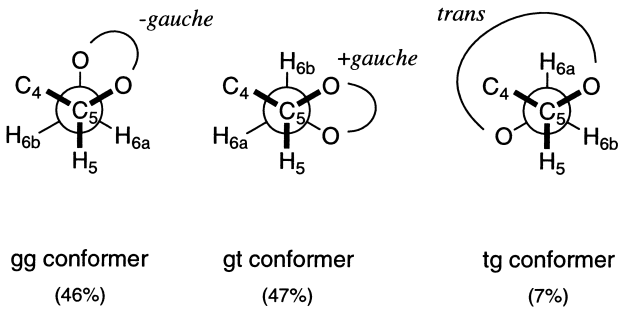


Fig. 6. Three rotamers staggered with respect to the C_5–C_6 bond in Newman projection. Ratios are calculated from $^3J_{H5,H6a}$ and $^3J_{H5,H6b}$ values on the basis of Meguro's equation.[14]

$$^3J_{H5,H6a} = 1.3Pg^- + 2.7Pg^+ + 11.7Pt \quad (1)$$

$$^3J_{H5,H6b} = 1.3Pg^- + 11.5Pg^+ + 5.8Pt \quad (2)$$

$$Pg^- + Pg^+ + Pt = 1 \quad (3)$$

The $^2J_{C6,H5}$ value (−2.3 Hz) also supports this consideration that both gg and gt conformers exist, because it fits neither of the typical values[21] reported for the gg (0–+2 Hz) and gt (−4–−5 Hz) conformers in the case of glycol connectivities.

The $^3J_{C6,H1'}$ value was used to determine the dihedral angle (ϕ) of the C_6–O_6–$C_{1'}$–$H_{1'}$ moiety according to the Karplus equation with Tvaroska's coefficients (Eq. 4):[22]

$$^3J_{C,H} = 5.7\cos^2\theta - 0.6\cos\theta + 0.5 \quad (4)$$

From Eq. 4, the representative value for a *gauche* conformer is ca. 2 Hz, and that for a *trans* conformer is over 6 Hz. The medium value of $^3J_{C6,H1'}$ (4.6 Hz) in the present study would either be an average value of several staggered conformers (Fig. 7), or would arise from one restricted conformation, wherein the dihedral angle of C_6-O_6-$C_{1'}$-$H_{1'}$ is ±25° or ±143° (Fig. 8). In the former case, we should consider the presence of three conformers (**A**, **B**, and **C**). In the *trans* conformer **A** and −*gauche* conformer **B**, NOEs should be observed from $H_{2'}$ to one of H_6 or other protons on the reducing-side glucosamine. They were, however, not observed at all with various mixing times at 500 and 600 MHz, either (Fig. 5). Additionally, conformer **B** is too unstable to exist even in calculation studies, probably due to strong repulsion between C_6 and the 2′-amide group. Among these conformers, the remaining +*gauche* conformer **C** is the only possibility, which, however, is inconsistent with the above initial assumption of conformational equilibrium. We therefore considered that the value of $^3J_{C6,H1'}$ reveals the presence of only one conformer in Fig. 8, but the conformers **K**, **L**, and **M** can be excluded (like conformers **A** and **B** in Fig. 7) from the candidates by taking the above NOESY experiment into consideration again. It was thus concluded that **N** is the most popular conformer among those shown in Fig. 8, where the dihedral angle of C_6–O_6–$C_{1'}$–$H_{1'}$ is +25°. This conclusion is also supported by assuming the relative thermodynamic stability of rotamers: conformer **N** has a steric interaction only between $H_{1'}$ and C_6, whereas the other conformers have more repulsions between spatially near groups.

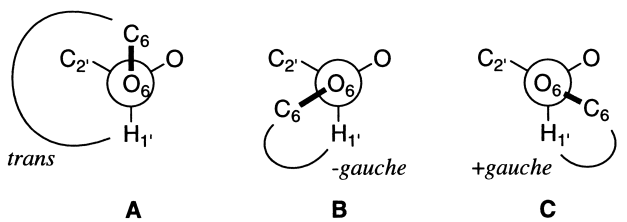


Fig. 7. Three rotamers staggered with respect to the O_6–$C_{1'}$ bond in Newman projection.

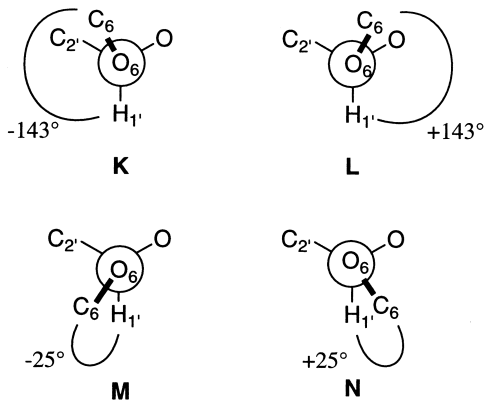


Fig. 8. Four rotamers, whose dihedral angles at C_6–O_6–$C_{1'}$–$H_{1'}$ satisfy the Karplus equation (Eq. 4) with Tvaroska's coefficients,[22] with respect to O_6–$C_{1'}$ bond in Newman projection.

Because a conformational analysis at the C_6–O_6 bond is not supported by the spin-coupling constant, a conclusion was drawn from the NOE data. Conformers **Y** (+*gauche*) and **Z** (−*gauche*) among three staggered conformers shown in Fig. 9 can be excluded, because no NOEs were observed from $H_{1'}$ to any protons other than two H_6 on the reducing-side saccharide. Strong NOEs at $H_{1'}$/H_{6a} and $H_{1'}$/H_{6b} also support the predominated existence of the **X** conformer over **Y** and **Z**. In addition, the *trans* conformer **X** has the weakest steric interactions between spatially near groups around $C_{1'}$.

The conformations of two amide protons, 2-NH and 2′-NH, were determined to be in favor of *trans* against H_2 or $H_{2'}$, respectively, because their $^3J_{H,NH}$ values (9.0 Hz for 2-NH and 6.0 Hz for 2′-NH) are rather large.

The conformations of the two phosphates could be determined by the $^3J_{P1,H1}$ (6.6 Hz) and $^3J_{P4',H4'}$ (6.4 Hz) values in consideration of the Karplus equation (Eq. 5),

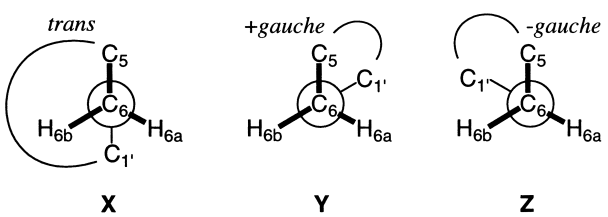


Fig. 9. Three rotamers staggered with respect to the C_6–O_6 bond in Newman projection.

$$^3J_{P,H} = A\cos^2\theta - B\cos\theta \quad (5)$$

wherein A and B are coefficients reported by several researchers[23] and θ is the dihedral angle of P–O–C–H. Because of the NOESY correlation between 2-NH and H_1 in Fig. 5, as well as the resonance of 2-NH at a higher-field (δ 7.32) than that of 2′-NH (δ 8.76) in a ^{1}H NMR spectrum, it seems that there is some kind of electronic interactions around 2-NH, which arrange in a *trans* conformation against H_2 (vide supra). The interaction would be between a somewhat positive 2-NH and the negative 1-phosphate to cause a conformational restriction around these functionalities. Indeed, by putting the $^3J_{P1,H1}$ value (6.6 Hz) into Eq. 5, the dihedral angle of P_1–O_1–C_1–H_1 was calculated to be +32.5° –+45°, which allows an ionic interaction between 2-NH and the 1-phosphate.

On the other hand, the conformation of the equatorial 4′-phosphate would not suffer a severe restriction by steric interactions with neighboring groups. In such a situation, $^3J_{P4',H4'}$ (6.4 Hz) should appear as an average value of several conformers, whose population ratio (Pt and Pg) can be determined by the following equations:

$$^3J_{P,H} = J\mathrm{t} \times \mathrm{Pt} + J\mathrm{g} \times \mathrm{Pg} \quad (6)$$

$$\mathrm{Pt} + \mathrm{Pg} = 1 \quad (7)$$

Jt and Jg are typical $^3J_{P,H}$ values reported[23] for the *trans* and *gauche* conformations, respectively, in P–O–C–H connectivity. From Eqs. 6 and 7, the relationship between $H_{4'}$ and $P_{4'}$ turned out to be in favor of *gauche* (*gauche*:*trans* = 85:15–77:23).

From these NMR analyses, the C_5–C_6 and $C_{4'}$–$O_{4'}$ bonds were found to be free from any rotational restriction, whereas the C_1–O_1, C_2–N_2, C_6–O_6, $C_{1'}$–O_6, and $C_{2'}$–$N_{2'}$ bonds are rather restricted. Because the arrangement of 4′-phosphate was shown to be preferentially *gauche*, only two conformational isomers (designated as GG and GT conformers in this paper), which differ only in the C_5–C_6 bond, were next submitted to individual computations.

Molecular Mechanics Calculations. The conformational information obtained from the NMR study is summarized in Fig. 10. From this data, the geometries of the GG and GT conformers were optimized by molecular mechanics calculations with Discover® of InsightII® software[24] using the cff91 force field. The geometry optimization was performed by a conjugate gradient algorithm with a dielectric value of DMSO (ε = 46.7)[25] until the convergence reached the derivative value of 0.001. Because the force field is not good for estimating the electronic structure, to both the GG and GT structures were applied conformational constraints during the calculations: two dihedral angles of P_1–O_1–C_1–H_1 (+33°) and $P_{4'}$–$O_{4'}$–$C_{4'}$–$H_{4'}$ (−60°), as well as the distance between 2-NH and 1-phosphate oxygen to be 1.76 Å, all of which had been estimated individually beforehand on a model compound at the PM3 level of theory.[17,18] The dihedral angles of C_6–O_6–$C_{1'}$–$H_{1'}$ (+25°) were also applied as a constraint condition. Additionally, the *s-trans* conformations were adopted for the ester and amide functionalities, and all carbon chains of fatty acid residues were in *trans* conformations in the initial structure for calculations. Two phosphate groups were treated as anions.

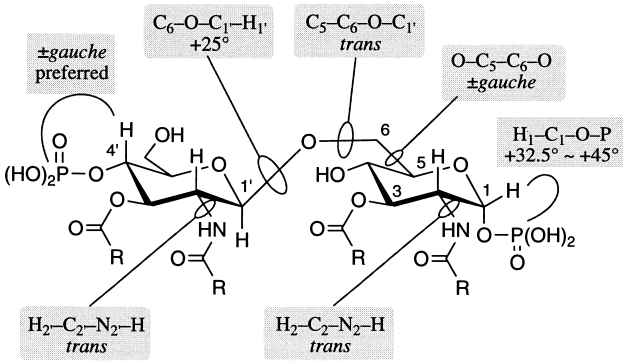


Fig. 10. The information for dihedral angles of **1** in DMSO obtained from NMR analysis. RCO = (*R*)-3-hydroxytetradecanoyl.

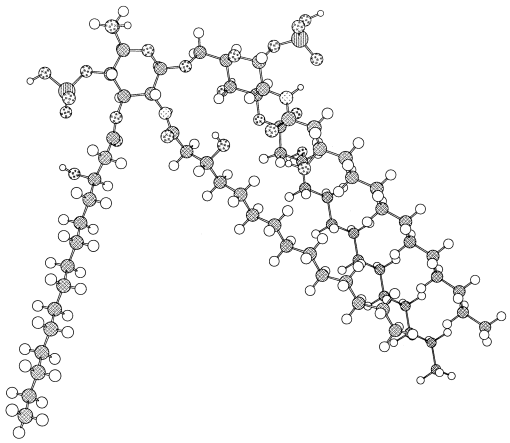


Fig. 11. The optimized GG conformer of **1** at the cff91 force field.

The optimized structures are shown in Fig. 11 (the GG conformer) and Fig. 12 (the GT conformer) in ball and stick drawings. The structures of the GG and GT conformers are similar to each other, except for the C_5–C_6 bond. The dihedral angles (ω) of O_5–C_5–C_6–O_6 are satisfactorily −64.0° for the GG conformer and +71.5° for the GT conformer (Table 2). Other important NMR and calculated data on the dihedral angles and the distances are also summarized in Table 2. The constrained dihedral angles around the O_6–$C_{1'}$ bond are well preserved after a calculation. The dihedral angles at C_5–C_6–O_6–$C_{1'}$, which have been concluded to be *trans* from the present NMR study (vide supra), were found to lie within the acceptable extents for a *trans* conformation (±150–180°); −154.2° for the GG and −162.8° for the GT conformers. As can also be seen from Figs. 11 and 12 that these two values are consistent with the NOEs for $H_{1'}$/H_{6a} and $H_{1'}$/H_{6b} observed in both conformers, though considerable differences are realized between the NMR data (2.66 Å for $H_{1'}$/H_{6a} and 2.69 Å for $H_{1'}$/H_{6b}) and the calculated data (more than 2.85 Å for $H_{1'}$/H_{6a} and less than 2.19 Å for $H_{1'}$/H_{6b}). An error in a NOESY build-up experiment, a computation error at the cff91 force field, and/or structural fluctuation at the C_6–O_6 bond could be possible reasons for these differences. Also, for a more precise discussion on the conformation around the C_6–O_6 bond, NMR data of the 1′-^{13}C-

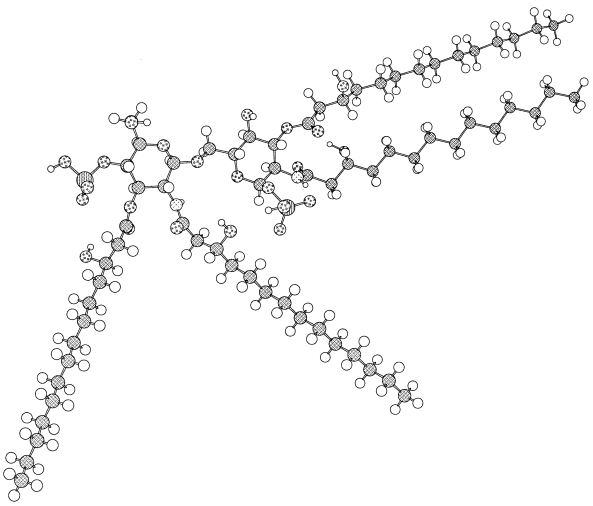


Fig. 12. The optimized GT conformer of **1** at the cff91 force field.

labeled specimen of tetraacyl lipid A (1′-^{13}C-**1**) are required, whose synthesis[26] has been recently achieved.

Table 2 also includes other important NMR and calculated data. The constrained dihedral angle around two phosphates are preserved after calculations. The initial *trans* conformation of amide protons, 2-NH and 2′-NH, against their vicinal protons is also well preserved. Owing to the distance constraint between 2-NH and 1-phosphate oxygen, the experimentally determined distances around 2-NH (2-NH/H_1 and 2-NH/H_3) are also reproduced.

From the above-mentioned NMR study, the biosynthetic precursor **1** is assumed to be present either in the GG or GT conformers in DMSO. The two conformers are considered to be interchanged at a faster rate than the time scale of NMR. Although the energy difference between the GG and GT conformers at the cff91 force field was evaluated to be 10.5 kcal mol^{-1}, the actual one at 30 °C can be estimated to be far less than 0.1 kcal mol^{-1} from the Boltzmann's distribution because the ratio of the rotamers (gg and gt) around the C_5–C_6 bond was determined to be 46%:47% by NMR. A smaller effect of the hydrophobic interactions[27] of the fatty acid residues to the conformation of the hydrophilic part of **1** would allow a nearly free conformational exchange between them in DMSO than in an aqueous medium, wherein **1** is present in an aggregated, rigid structure.[28]

Summary

The solution conformation of the biosynthetic precursor of lipid A in DMSO has been unambiguously determined by means of NMR analyses of both 6-^{13}C-labeled and nonlabeled **1** in DMSO-d_6. The enriched compound allowed us to determine the $^nJ_{C6,H}$ values, which have been never determined with a nonlabeled specimen. The spin-coupling constants of $^3J_{P1,H1}$, $^3J_{H2,2\text{-}NH}$, $^3J_{C6,H1'}$, $^3J_{H2',2'\text{-}NH}$, and $^3J_{P4',H4'}$ were used to determine the dihedral angles around the C_1–O_1, C_2–N_2, $C_{1'}$–O_6, $C_{2'}$–$N_{2'}$, and $C_{4'}$–$O_{4'}$ bonds, respectively. By applying the obtained $^3J_{H5,H6a}$ and $^3J_{H5,H6b}$ values to the empirical rule established by Meguro et al.,[14] two C_5–C_6 rotamers, gg and gt, were found to be present in an almost equal ratio. Each structure of the GG and GT conformers was then optimized by molecular mechanics calculations at the cff91 force field with some structural constraints, and well-optimized structures were obtained for both conformers.

The present discussion is reliable because the analyses were mainly performed based on the spin-coupling constants between two nuclei. This method now opens a way to conduct conformational analyses of lipid A analogues in biologically more important aqueous media in which lipid A hardly dissolves.[28] It should also be noted here that the present methodology would be generally applicable to conformational studies on other biologically important oligosaccharides, particularly highly flexible ones containing (1→6) linkages. A further

Table 2. Comparison of the Selected Geometry Obtained from NMR and Molecular Mechanics Calculations at the cff91 Force Field

	Experimental NMR data[a)]	GG conformer after calculation	GT conformer after calculation
Dihedral angles (degree)			
P_1–O_1–C_1–H_1[b)]	+32.5–+45	+34.3	+34.4
$P_{4'}$–$O_{4'}$–$C_{4'}$–$H_{4'}$[b)]	±*gauche*	−59.0	−59.1
O_5–C_5–C_6–O_6[ω]	±*gauche*	−64.0	+71.5
C_5–C_6–O_6–$C_{1'}$[ψ]	*trans*	−154.2	−162.8
C_6–O_6–$C_{1'}$–$H_{1'}$[ϕ][b)]	+25	+25.1	+25.1
H_2–C_2–N_2–H	*trans*	−159.5	−156.7
$H_{2'}$–$C_{2'}$–$N_{2'}$–H	*trans*	−154.2	−147.0
Distances (Å)			
(2-N<u>H</u>)/H_1	2.85	2.96	2.91
(2-N<u>H</u>)/H_3	2.74	2.95	3.00
$H_{1'}$/H_{6a}	2.66	3.05	2.85
$H_{1'}$/H_{6b}	2.69	2.18	2.19

a) In the column of the NMR data, the dihedral angles were from $^nJ_{X,H}$ values, whereas the distances were obtained from NOESY build-up experiments.
b) Rotating angles were constrained during the calculation. See text.

NMR study on a complex of **1** and a binding small peptide[29] in DMSO is now in progress.

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