Structural Studies on Fortimicins. III. The Crystal and Molecular Structure of Fortamine Dihydrochloride

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Fortamine dihydrochloride, $C_8H_{18}N_2O_4 \cdot 2HCl$, is obtained by the hydrolysis of fortimicin B with hydrochloric acid. The space group is *C2* with $a=16.109(2)$, $b=10.047(1)$, $c=8.603(1)$ Å, $\beta=108.69(1)°$, and $Z=4$. The structure was solved by the heavy atom method, and least-squares refinement using 1564 reflexions led to the final R value of 0.030. The ring conformation of fortamine dihydrochloride is an inversed chair form of fortamine. The chair form is significantly compressed to relax the non-bonded interactions between axial substituents.

In the studies on the structures of fortimicins, potent aminocyclitol antibiotics, Egan *et al.* have reported that the ring conformation of 1,4-diaminocyclitol in fortimicin B must be inversed when its amino groups are protonated in its sulfate.[1] Similar unusual conformational inversion occurs in fortimicin A (4-*N*-glycylfortimicin B).[1] We have achieved X-ray analyses of fortimicin B[2] and fortamine,[3] and found that 1,4-diaminocyclitol rings take a normal conformation as shown in Fig. 1. We will denote this conformation as 'B' type and inversed one as 'A' type. Because of the inherent 1,3-diaxial interactions, 'A' type conformation seems to be unstable and it is necessary to examine the validity of the assignment by Egan *et al.*[1] Unfortunately, attempts to crystallize fortimicin B sulfate and fortimicin A were unsuccessful, but crystals of fortamine dihydrochloride were obtained. Its structure determination would throw light on the effect of protonation on the ring conformation.

Experimental and Structure Determination

Fortamine dihydrochloride was obtained by the hydrolysis of fortimicin B with hydrochloric acid. The prismatic crystals were grown from a methanol solution. A crystal of $0.5\times0.4\times0.3$ mm^3 sealed in a glass capillary was used for data collection on a Rigaku four-circle automated diffractometer with graphite monochromated Mo $K\alpha$ radiation ($\lambda=0.71069$ Å). Preliminary unit-cell dimensions and space group were obtained from photographs. The space group was determined from the systematic absences (*hkl* for $h+k=2n+1$). Accurate cell dimensions were determined by least-squares calculation with the 2θ values of 15 high angle reflexions measured on the diffractometer. Crystal data are summarized in Table 1. All reflexions within the range of $2\theta\leqq55°$ were collected by the use of the ω-2θ scan mode with a scanning rate of $4°(2\theta)$ min^{-1}. Stationary background counts were accumulated for 10 s before and after each scan. Periodic checks of the intensity values of three standard reflexions did not reveal any significant X-ray damage or crystal decay. Any corrections for absorption or extinction were not applied. A total of 1610 independent reflexions were obtained, of which 1564 ($|F_o|\geqq3.0\sigma(|F_o|)$) were considered as observed.

The structure was solved by the heavy atom methods and refined by the block-diagonal least-squares methods with the modified HBLS program. All the hydrogen atoms were found by the difference Fourier synthesis. Refinement was brought to completion using anisotropic and isotropic thermal parameters for the non-hydrogen and hydrogen atoms, respectively. In the later stage of the refinement, the following weighting system was adopted;
$w=0.3$ for $|F_o|<3.72$ and $|F_o|>37.16$, and

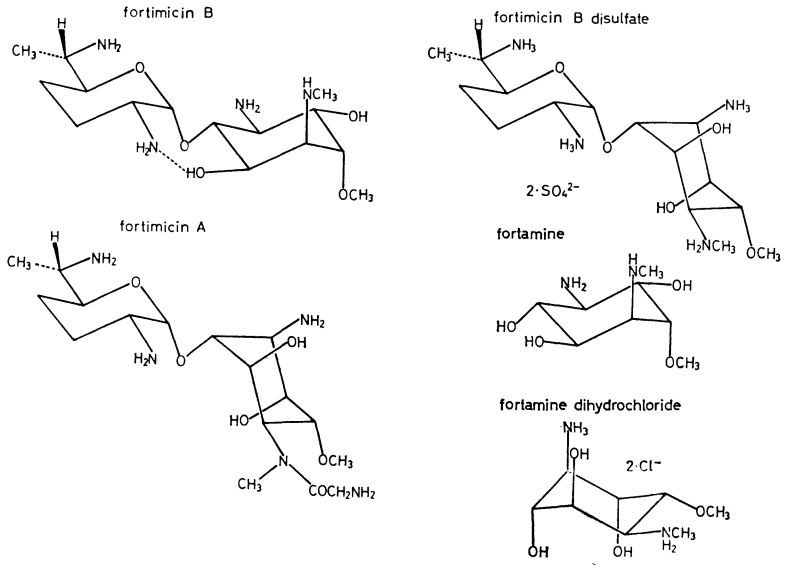

Fig. 1. Structures of fortimicins and fortamines.

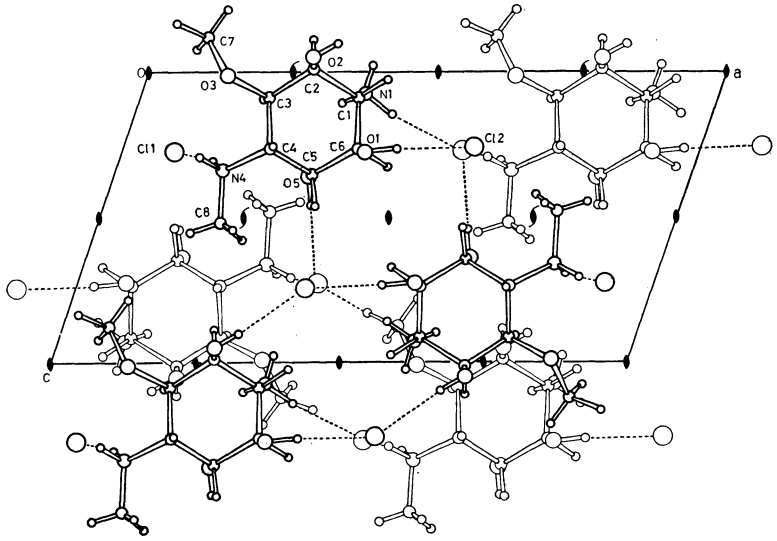

Fig. 2. The crystal structure projected along the b axis.

Table 1. Crystal data

$C_8H_{20}N_2O_4Cl_2$	$b=10.047(1)$
M.W.=277.15	$c=8.603(1)$ Å
C2	$\beta=108.69(1)°$
$Z=4$	$D_x=1.396$ g cm^{-3}
$a=16.109(2)$	μ(Mo $K\alpha$) $=4.961$ cm^{-1}

$w=(0.00833(F_o)^2-0.34114|F_o|+4.48559)^{-1}$ for $3.72\leqq|F_o|\leqq37.16$. The final R value was 0.030. Atomic scattering factors were taken from "International Tables for X-Ray Crystallography."[4] Differences in the intensities of six Friedel pairs of reflexions measured on a diffractometer using Cu $K\alpha$ radiation confirmed the absolute configuration previously determined by CD measurements.[1] The final positional and thermal parameters are given in Table 2. A list of the observed and calculated structure factors is kept as Document No. 8035 at the Chemical Society of Japan.

Results

Hydrogen Bond and Crystal Structure. The crystal structure viewed along the b axis is shown in Fig. 2. Hydrogen bond lengths and angles are listed in Table 3. All functional groups are involved in intermolecular hydrogen bonds with the chloride anions, but there is no hydrogen bond between functional groups. The N(4) atom makes bifurcated hydrogen bonds with Cl(1) and Cl(2). Two short contacts are found between O(5) and N(1), and between O(3) and N(4); O(5)···N(1) and O(3)···N(4) distances being 2.802 and 2.703 Å, and O(5)···H–N(1) and O(3)···H–N(4) angles 109.2 and 97.8°, respectively. Judging from these geometries, it is not plausible to assign hydrogen bonding to these interactions.

Bond Lengths and Angles. The bond lengths and angles are shown in Table 4, in which corresponding values of fortamine are also given. The average endocyclic C–C bond length of 1.527 Å in the dihydrochloride is essentially equal to that of 1.524 Å in fortamine. This indicates that protonation at the amino groups hardly affects the endocyclic C–C bond lengths. Although the C(1)–C(2) length of fortamine is significantly short (1.505 Å), that of the dihydrochloride (1.542 Å) is notably long. The C(1)–N(1), C(4)–N(4) and N(4)–C(8) bond lengths of the dihydrochloride becomes longer than those of fortamine under the influence of protonation. All C–O (hydroxyl) bond lengths except C(5)–O(5) are significantly shorter than those of fortamine. The C(2)–O(2) bond length (1.411 Å) is particularly short.

The average endocyclic C–C–C bond angles is 112.1°. This value is larger than 111.4° of fortamine. The effect of protonation is seen in the endocyclic bond angles, too. In epi-inositol, the C–C–C angle between 1,3-diaxial hydroxyl group is 114.7°.[5] Similar 1,3-diaxial interaction between O(1) and O(2) (O(1)···O(2) distance being 3.01 Å) in the present molecule widen the C(6)–C(1)–C(2) angle to 114.3°. On the contrary, the C(1)–C(6)–C(5) angle of 112.4(2)° is not so large and does not seem to suffer from the N(1)···O(5) non-bonded interaction, although the N(1)···O(5) distance is shorter (2.820 Å).

All of the exocyclic bond angles except O(3)–C(3)–C(4) and C(7)–O(3)–C(3) in the dihydrochloride are significantly different from those in the free base, fortamine. The bond angles around N(4)–C(4) and O(3)–C(3) are remarkably asymmetrical. This seems to indicate that there is some attractive force between N(4) and O(3).

Conformation of the Molecule. The ring conformation of fortamine dihydrochloride, as shown in Fig. 3, is inverse of the free base conformation. The signs of torsional angles listed in Table 5 also represent the ring inversion. Figure 4 shows the important torsional angles between substituents and the d values, defined by deviations of the two atoms from the least-squares plane through other four atoms consisting of the seat of a chair. The average C–C–C–C torsional angles in the ring is 52.7° and average d is 0.627 Å. Both of these values are smaller than those of the free base, 54.9° and 0.655 Å, respectively. The ring of the dihydrochloride is flattened to some extent

Table 2a. Final atomic coordinates with their estimated standard deviations, multiplied by 10^5 for Cl atoms, 10^4 for C, N, and O atoms, and 10^3 for H atoms

Atom	x	y	z	B_{eq}[a] or B	Atom	x	y	z	B_{eq}[a] or B
C(1)	1250(2)	1949(2)	9154(3)	2.18	HC(3)	289(2)	108(3)	911(3)	2.1
C(2)	2198(2)	2355(3)	1012(3)	2.08	HC(4)	246(2)	355(3)	742(3)	2.1
C(3)	2821(1)	2066(2)	9154(3)	1.87	HC(5)	135(2)	235(4)	538(3)	3.2
C(4)	2487(1)	2596(2)	7406(3)	1.82	HC(6)	34(2)	197(3)	672(3)	2.3
C(5)	1592(2)	2033(3)	6470(3)	2.09	HAC(7)	430(3)	109(5)	1086(6)	8.3
C(6)	940(2)	2397(3)	7349(3)	2.26	HBC(7)	403(2)	181(4)	1215(5)	5.7
C(7)	4215(2)	1886(4)	11223(4)	3.86	HCC(7)	483(2)	231(5)	1166(4)	5.4
C(8)	2911(2)	2395(4)	4807(3)	3.33	HAC(8)	243(2)	169(3)	423(4)	3.3
N(1)	1165(1)	461(2)	9256(3)	2.24	HBC(8)	340(2)	240(4)	450(4)	3.9
N(4)	3169(1)	2242(3)	6619(2)	2.09	HCC(8)	266(2)	326(3)	453(4)	3.5
O(1)	840(1)	3798(2)	7259(3)	3.36	HAN(1)	59(2)	18(4)	860(4)	4.2
O(2)	2263(1)	3721(2)	10525(3)	3.07	HBN(1)	117(2)	26(3)	1026(3)	2.2
O(3)	3662(1)	2638(2)	9897(2)	2.27	HCN(1)	157(2)	2(4)	900(4)	3.7
O(5)	1667(1)	618(2)	6409(2)	2.43	HAN(4)	365(2)	276(3)	708(3)	1.9
Cl(1)	41486(4)	50057(8)	72985(7)	2.73	HBN(4)	340(2)	131(4)	692(4)	4.5
Cl(2)	−10356(4)	43746(9)	74524(9)	3.11	HO(1)	29(2)	399(4)	732(5)	4.6
HC(1)	87(2)	229(3)	974(3)	2.4	HO(2)	193(2)	386(4)	1102(4)	3.8
HC(2)	238(2)	190(3)	1114(3)	2.6	HO(5)	141(2)	35(4)	541(4)	4.1

a) $B_{eq}=\frac{1}{3}(2\pi^2)^{-1}\sum_i\sum_j \beta_{ij}\boldsymbol{a}_i\cdot\boldsymbol{a}_j$, here $\boldsymbol{a}_i$ and $\boldsymbol{a}_j$ are the base vectors in real space, and β_{ij}'s are anisotropic thermal parameters.[8]

Table 2b. Final thermal parameters with their estimated standard deviations. Anisotropic thermal parameters are multiplied by 10^4 and isotropic ones multiplied by 10^3

Atom	U or U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(1)	274(11)	230(13)	373(12)	−15(9)	181(10)	−30(9)
C(2)	333(11)	215(12)	278(10)	−48(10)	148(9)	−33(10)
C(3)	227(10)	189(11)	279(10)	−18(8)	64(8)	−14(9)
C(4)	243(10)	199(10)	279(10)	14(8)	132(9)	2(8)
C(5)	257(10)	255(13)	268(10)	0(9)	65(9)	15(9)
C(6)	224(10)	238(13)	408(13)	21(9)	116(10)	40(10)
C(7)	362(15)	515(20)	481(16)	−10(13)	−34(13)	116(14)
C(8)	427(14)	582(20)	298(12)	−31(14)	176(11)	8(14)
N(1)	293(10)	261(10)	327(10)	−62(8)	143(9)	7(9)
N(4)	250(9)	282(9)	288(9)	−21(9)	127(8)	−17(9)
O(1)	377(10)	261(9)	707(14)	109(8)	281(11)	117(10)
O(2)	476(11)	260(9)	539(11)	−105(8)	325(10)	−174(9)
O(3)	238(7)	261(9)	329(8)	−44(7)	40(7)	6(7)
O(5)	362(9)	243(8)	299(9)	−28(8)	80(8)	−51(7)
Cl(1)	346(3)	367(3)	292(2)	−111(3)	62(2)	40(3)
Cl(2)	349(3)	337(3)	564(4)	47(3)	248(3)	6(3)
HC(1)	28(7)					
HC(2)	31(7)					
HC(3)	24(7)					
HC(4)	25(7)					
HC(5)	38(8)					
HC(6)	29(7)					
HAC(7)	122(19)					
HBC(7)	72(13)					
HCC(7)	73(11)					
HAC(8)	43(9)					
HBC(8)	51(9)					
HCC(8)	44(9)					
HAN(1)	53(9)					
HBN(1)	28(7)					
HCN(1)	47(9)					
HAN(4)	23(7)					
HBN(4)	55(10)					
HO(1)	67(11)					
HO(2)	45(9)					
HO(5)	52(10)					

from the free base. As seen from the *d* values, C(4) and C(5) are puckered and C(1) and C(2) flattened. Small C(5)–C(4)–N(4)–C(8) torsional angle of 44.8° is probably due to the bifurcated hydrogen bond of N(4). Some exocyclic torsional angles between axial substituents in the dihydrochloride are fairly larger than those in the free base. This indicates the exocyclic torsional angles also suffer from 1,3-diaxial interaction to a large extent. Therefore, the smallest exocyclic O(2)–C(2)–C(3)–O(3) torsional angle of 46.5° is due to the O(1)···O(2) repulsion.

Table 3. Distances and angles of hydrogen bond A-H···B

	l(A···B)/Å	ϕ(A-H···B)/°
N(4)···Cl(1)^{I}	3.155(3)	141.9
O(1)···Cl(2)^{I}	3.132(3)	175.5
N(4)···Cl(2)^{II}	3.141(3)	175.6
N(1)···Cl(1)^{III}	3.176(2)	165.7
O(5)···Cl(1)^{IV}	3.096(2)	169.8
O(2)···Cl(2)^{V}	3.094(2)	174.3

Symmetry code

I:	(x	y	z)
II:	($1/2+x$	$-1/2+y$	z)
III:	($-1/2+x$	$-1/2+y$	z)
IV:	($1/2-x$	$-1/2+y$	$1-z$)
V:	($-x$	y	$2-z$)

Discussion

Fortamine dihydrochloride takes the '*A*' type conformation in the crystal structure. This result shows that the protonation at the two nitrogen atoms makes

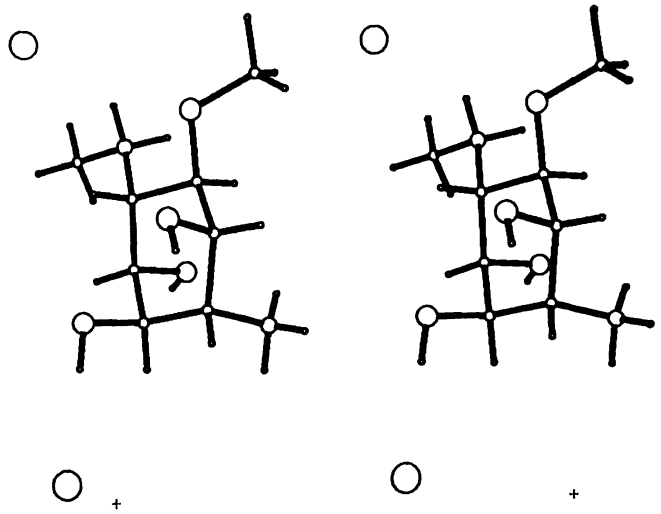

Fig. 3. A stereoscopic drawing of the molecule. This was drawn by TSD: XTAL, which is a computer-graphics interactive modeling program for NOVA 3 computer.[7]

Table 4. Bond lengths (l/Å) and angles (ϕ/°) with their estimated standard deviations in parentheses. Bond angles including the hydrogen atoms are omitted for clarity

	Fortamine dihydrochloride	Fortamine		Fortamine dihydrochloride	Fortamine
C(1)-C(2)	1.542(4)	1.505(4)	O(5)-HO(5)	0.89(4)	0.92(3)
C(2)-C(3)	1.523(4)	1.513(4)	C(7)-HAC(7)	0.92(5)	0.96(4)
C(3)-C(4)	1.521(3)	1.532(4)	C(7)-HBC(7)	0.93(5)	1.11(4)
C(4)-C(5)	1.518(4)	1.528(4)	C(7)-HCC(7)	1.04(5)	0.92(5)
C(5)-C(6)	1.522(4)	1.521(4)	C(8)-HAC(8)	1.06(4)	0.98(3)
C(6)-C(1)	1.538(4)	1.545(4)	C(8)-HBC(8)	0.90(4)	1.05(4)
C(1)-N(1)	1.506(4)	1.470(4)	C(8)-HCC(8)	0.94(4)	1.00(3)
C(2)-O(2)	1.411(3)	1.432(3)	C(1)-C(2)-C(3)	111.5(2)	112.6(2)
C(3)-O(3)	1.421(3)	1.434(4)	C(2)-C(3)-C(4)	112.8(2)	111.1(2)
O(3)-C(7)	1.420(4)	1.414(4)	C(3)-C(4)-C(5)	111.7(2)	109.9(2)
C(4)-N(4)	1.506(4)	1.485(4)	C(4)-C(5)-C(6)	109.7(2)	112.0(2)
N(4)-C(8)	1.487(5)	1.469(4)	C(5)-C(6)-C(1)	112.4(2)	111.2(2)
C(5)-O(5)	1.430(3)	1.429(3)	C(6)-C(1)-C(2)	114.3(2)	111.4(2)
C(6)-O(1)	1.417(4)	1.434(3)	N(1)-C(1)-C(2)	108.9(2)	110.4(2)
C(1)-HC(1)	0.97(4)	0.92(3)	N(1)-C(1)-C(6)	110.0(2)	108.7(2)
C(2)-HC(2)	0.94(3)	0.99(3)	O(1)-C(6)-C(1)	109.9(2)	109.1(2)
C(3)-HC(3)	1.01(3)	1.05(3)	O(1)-C(6)-C(5)	107.2(2)	109.1(2)
C(4)-HC(4)	0.96(3)	1.05(3)	O(5)-C(5)-C(6)	109.7(2)	112.5(2)
C(5)-HC(5)	0.95(4)	1.08(3)	O(5)-C(5)-C(4)	108.2(2)	112.0(2)
C(6)-HC(6)	1.03(3)	1.04(3)	N(4)-C(4)-C(5)	112.3(2)	110.3(2)
N(1)-HAN(1)	0.97(4)	1.03(3)	N(4)-C(4)-C(3)	106.5(2)	111.8(2)
N(1)-HBN(1)	0.87(3)	0.89(3)	C(8)-N(4)-C(4)	116.6(2)	112.2(2)
N(1)-HCN(1)	0.89(4)		O(3)-C(3)-C(4)	106.7(2)	107.2(2)
N(4)-HAN(4)	0.92(3)	0.94(3)	O(3)-C(3)-C(2)	112.1(2)	109.6(2)
N(4)-HBN(4)	1.02(4)		C(7)-O(3)-C(3)	114.2(2)	114.5(2)
O(1)-HO(1)	0.93(4)	0.82(3)	O(2)-C(2)-C(3)	108.1(2)	110.7(2)
O(2)-HO(2)	0.81(4)	0.83(3)	O(2)-C(2)-C(1)	111.9(2)	108.7(2)

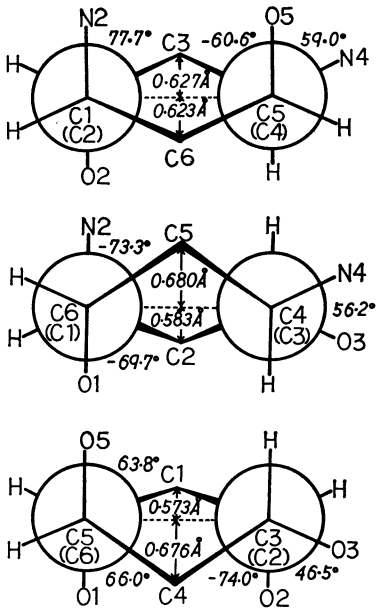

Fig. 4. Schematic drawings of chair forms of fortamine and fortamine dihydrochloride.

the 'B' type conformation unstable and inverse the fortamine ring into the 'A' type conformation. It seems that the electrostatic repulsion between the charge at the amino groups is responsible for the ring inversion. We calculated the electrostatic repulsion energies of the fortamine dihydrochloride in the 'A' type and in the 'B' type conformations. To simplify the treatment 1 e.s.u. charge due to protonation is assumed to be localized at each nitrogen atom. The geometry of the 'B' type dihydrochloride is taken as that of fortamine except the C–N distances which are replaced by the values observed in fortamine dihydrochloride. The calculation indicates that the electrostatic repulsion energy in the 'B' type conformation is 0.50 kcal/mol higher than that in the 'A' type conformation.

In the 'A' type conformation, however, the non-bonded interactions between the axial substituents seem to be more crucial than those in the 'B' type conformation. We estimated them by calculating the non-bonded interaction energy between the axial substituents. Giglio's potentials[6] were used and the effect of axial hydrogen atoms were also included. The value obtained was 1.19 kcal/mol. This is not so high; in other words, the non-bonded interactions between the axial substituents in the 'A' type conformation are fully relaxed by deforming the endocyclic angles and torsional angles as described in the previous section. Although further studies on the energy of molecular deformation would be required, it is most probable that the electrostatic force is ineviatble to stabilize fortamine dihydrochloride molecule in the 'A' type conformation. The inversed chair form significantly compressed to relax the 1,3-diaxial interactions would serve the basis to construct the structure of fortimicin A.

Table 5. Torsional angles (ϕ/°)

	Fortamine dihydrochloride	Fortamine
C(1)-C(2)-C(3)-C(4)	49.4	−56.1
C(2)-C(3)-C(4)-C(5)	−57.4	56.3
C(3)-C(4)-C(5)-C(6)	59.0	−56.4
C(4)-C(5)-C(6)-C(1)	−54.9	54.6
C(5)-C(6)-C(1)-C(2)	49.6	−52.6
C(6)-C(1)-C(2)-C(3)	−45.8	53.9
N(1)-C(1)-C(2)-O(2)	−161.1	−62.2
O(2)-C(2)-C(3)-O(3)	46.5	−59.8
C(2)-C(3)-O(3)-C(7)	80.6	129.2
C(4)-C(3)-O(3)-C(7)	−155.4	−110.1
O(3)-C(3)-C(4)-N(4)	56.2	173.9
C(3)-C(4)-N(4)-C(8)	167.3	−70.9
C(5)-C(4)-N(4)-C(8)	44.8	166.5
N(4)-C(4)-C(5)-O(5)	59.0	−60.1
O(5)-C(5)-C(6)-O(1)	−175.3	−57.9
O(1)-C(6)-C(1)-N(1)	167.4	65.2

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