Acta Crystallographica Section E
Structure Reports Online
ISSN 1600-5368

A β-sheet structure formed by C–H···O hydrogen bonds between the thiazole rings and amide bonds of a dimeric desoxazoline ascidiacyclamide analogue

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Received 17 July 2001
Accepted 30 July 2001
Online 10 August 2001

A dimeric analogue of desoxazoline ascidiacyclamide was synthesized to increase the conformational flexibility of the molecule. The overall structure of this compound, $C_{72}H_{112}N_{16}O_{16}S_4\cdot 3C_3H_7NO\cdot H_2O$, was relatively flat and was classified neither as the folded nor squared form, which have been observed in ascidiacyclamide analogues. Rather, a unique β-sheet was formed in the 48-membered ring with pseudo-twofold symmetry. This was stabilized by hydrogen bonds including C—H···O interactions between thiazole and carbonyl O atoms. This is the first structure of an ascidiacyclamide analogue to exhibit a flat conformation composed of a β-sheet.

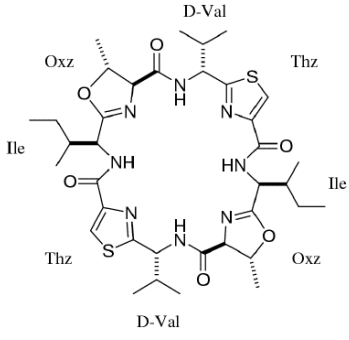
Key indicators

Single-crystal synchrotron study
T = 100 K
Mean σ(C–C) = 0.015 Å
Disorder in main residue
R factor = 0.097
wR factor = 0.259
Data-to-parameter ratio = 8.0

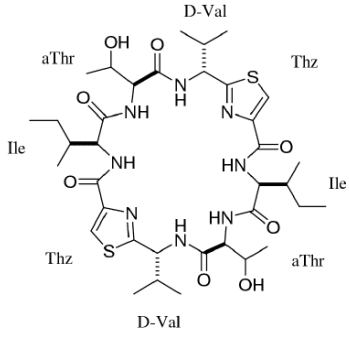
For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

Comment

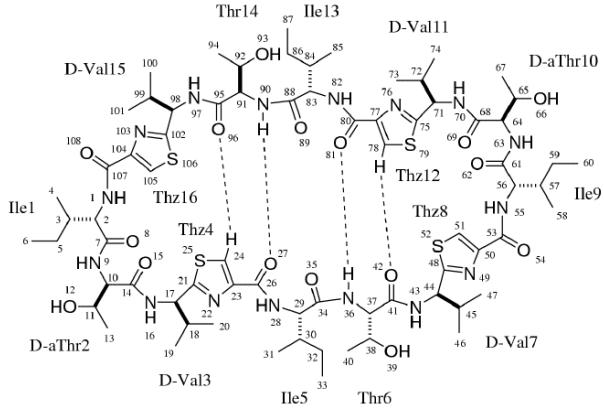
Ascidiacyclamide (ASC) is a cytotoxic peptide isolated from marine organisms (Ireland *et al.*, 1982; Hamamoto *et al.*, 1983),



Ascidiacyclamide (ASC)



desoxazoline-ascidiacyclamide (dASC)



desoxazoline-ascidiacyclamide dimer (dASC2)

and includes unusual amino acids, thiazole (Thz) and oxazoline (Oxz). Two major structures are known for this peptide

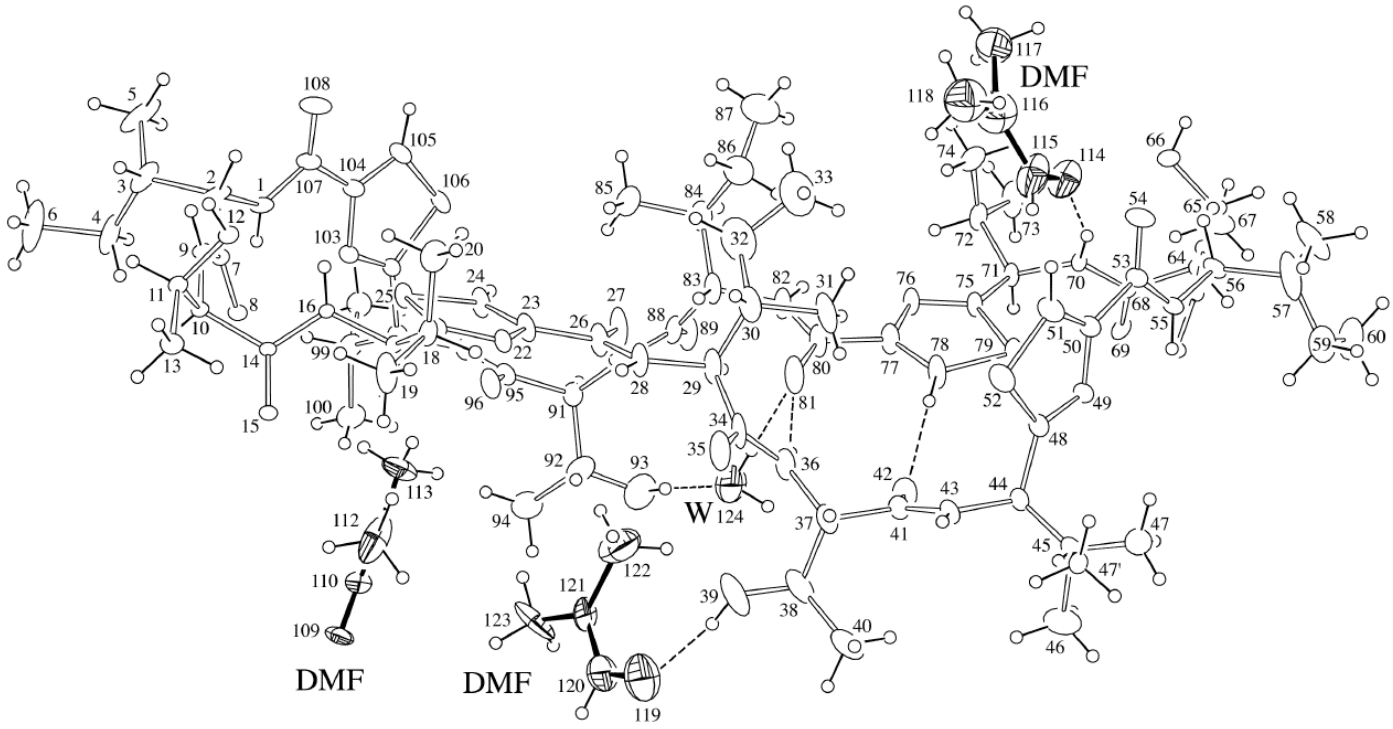


Figure 1
A view of dASC2 with the atomic numbering scheme. Displacement ellipsoids are drawn at the 40% probability level. Dashed lines represent hydrogen bonds. The solvent molecules are shaded.

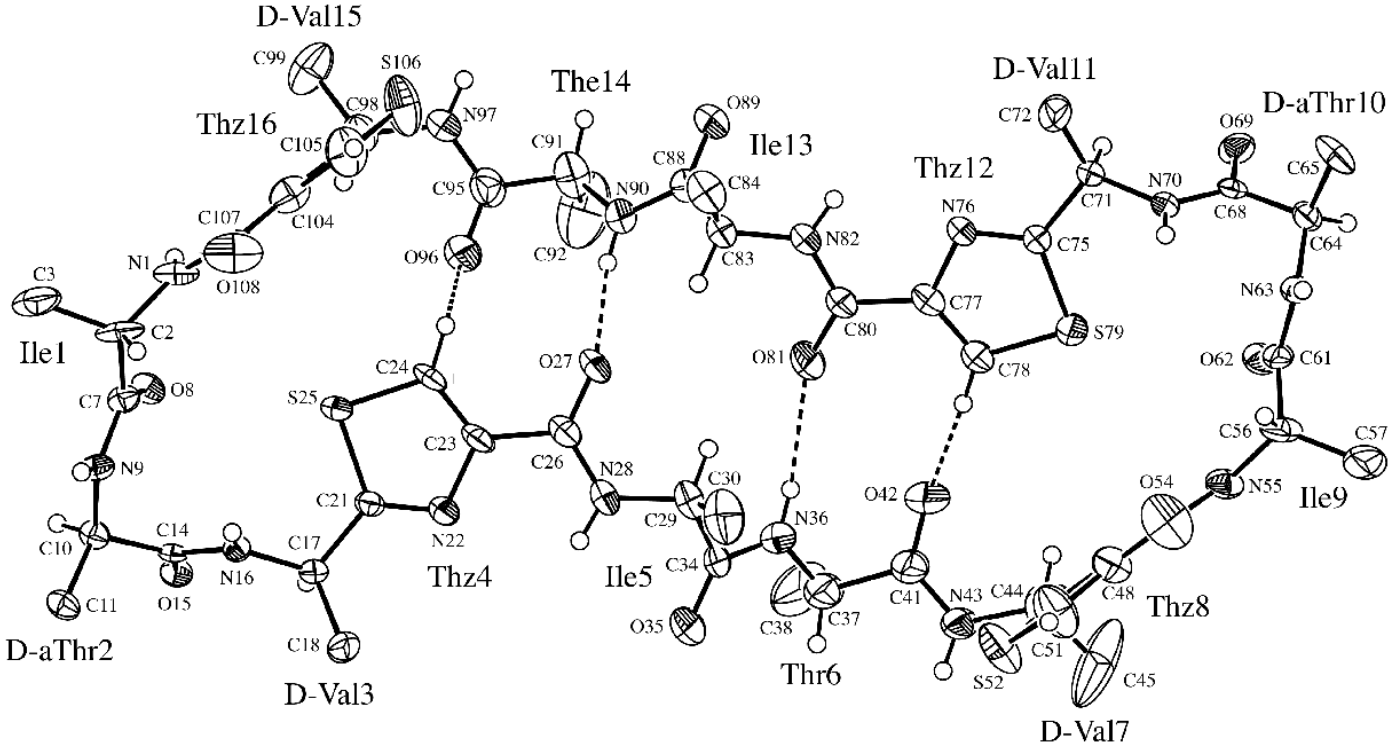


Figure 2
Top view of the peptide backbone. Side chains have been omitted for clarity (Cβ atoms only are drawn). Dashed lines represent hydrogen bonds.

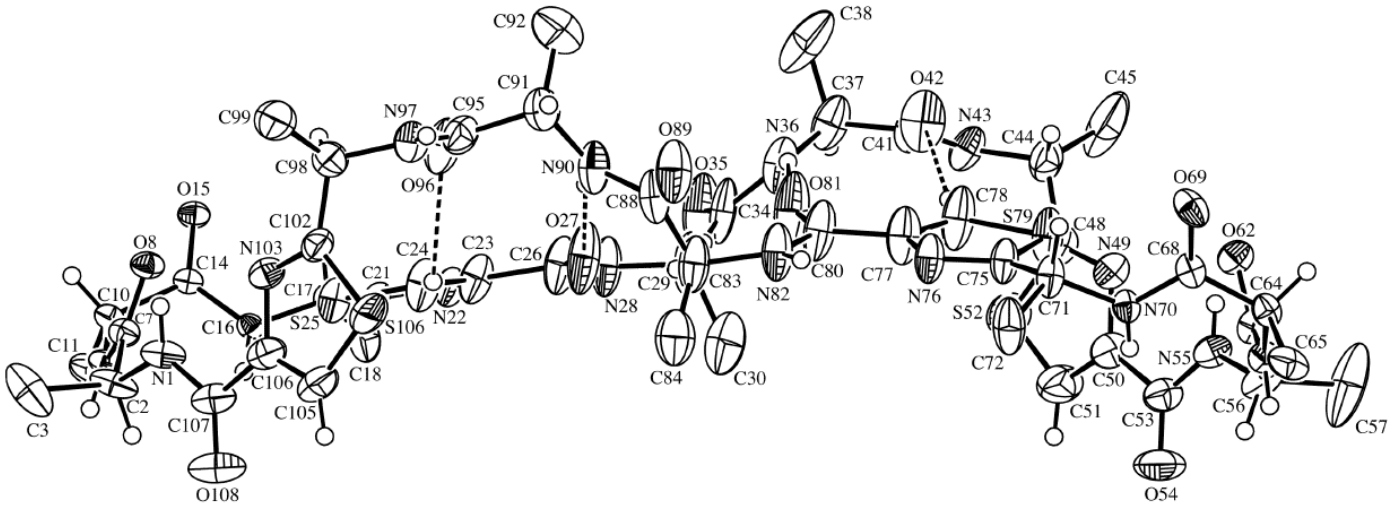


Figure 3
Side view of the peptide backbone. Side chains have been omitted for clarity (Cβ atoms only are drawn).

(Ishida *et al.*, 1989). We have attempted to control its structure by chemical modification, *e.g.* substitution of amino acids or changing the chirality (Doi *et al.*, 1999). The conformations of ASC analogues seem to be restricted by the rigid blocks composed of Thz and Oxz, since these blocks limit the rotations of peptide backbones (N—Cα and Cα—C bonds). Therefore, we tried to break the Oxz blocks to increase backbone flexibility. The Oxz block is synthesized from threonine and modification would result in threonine residues in place of the Oxz blocks. Threonine has two chiral C atoms at the α and β positions, and both C atoms could be targets of the chiral modifications (a diastereomer of threonine at the Cβ position is named *allo*-threonine, aThr). Such desoxazoline–ASC analogues (dASC) prefer a single conformation, *viz*. a folded form (Asano *et al.*, 2001). It was postulated that the molecular size of these cyclic peptides (24-membered ring) could limit the conformational variations.

Thus, we attempted a dimerization of these dASC diastereomers to give more conformational freedom to the peptide backbone. The dimeric desoxazoline–ASC (dASC2) analogues have a 48-membered ring in place of the 24-membered ring of ASC in dASC analogues. Crystals of [Thr,D-aThr]-dASC2 were obtained from the dASC2 diastereomers. The structure of this analogue is completely different from the folded or squared structure of ASC and a related peptide, patellamide (Schmitz *et al.*, 1989). We report here the crystal structure of [D-aThr,Thr]-dASC2.

Crystals of [D-aThr,Thr]-dASC2 were grown in aqueous dimethylformamide (DMF) solution and the X-ray diffraction data were measured on a synchrotron source. The structure is shown in Fig. 1. The peptide is slightly twisted, but no large folding is observed. In dASC analogues, the peptide molecules are folded such that the Thz rings face each other (Asano *et al.*, 2001), and the Thz–Thz stacking is a notable feature of a folded dASC. In this structure, the four Thz rings are separated from each other and no stacking is observed. The Thz4 and Thz12 rings are both roughly perpendicular to the Thz8 and Thz16 rings; the angles between the least-squares planes are Thz4···Thz8 = 85.7°, Thz4···Thz16 = 84.2°, Thz12···Thz8 = 78.2° and Thz12···Thz16 = 76.9°. The conformation of the peptide backbone is shown in Figs. 2 and 3. The peptide backbone forms a unique β-sheet structure (Fig. 2). In this β-sheet, C—H···O hydrogen bonds are formed between the Thz and carbonyl O atoms (Table 2): C24(Thz4)···O96(Thr14) = 3.158 (11) Å and C78(Thz12)···O42(Thr6) = 3.190 (12) Å. Such hydrogen bonds lead to a parallel arrangement of Thz4 and Thz12; the angle between the least-squares planes of Thz4 and Thz12 is 23.2°. Consequently, the 48-membered ring is relatively flat (Fig. 3). Furthermore, the peptide has a repeated sequence, and structural symmetry is found in the torsion angles of the backbone (Table 1). Although this implies the existence of a pseudo-twofold axis in the molecule, the crystallographic symmetry is distorted by local conformations and interactions with the solvent molecules; O39(Thr6)···O119(DMF) = 2.579 (24) Å, N70(D-Val11)···O114(DMF) = 2.963 (13) Å, N16(D-Val3)···O109(DMF) = 2.965 (9) Å and O93(Thr14)···O124(W) = 2.717 (22) Å.

The conformational properties which were found in the folded and squared structures of ASC and dASC analogues are not found in the structure of [D-aThr,Thr]-dASC2. The molecule is relatively flat, with accompanying slight twisting, and a unique β-sheet is formed, involving C—H···O hydrogen bonds. Dimerization gives conformational flexibility to the peptide molecule, and the resulting novel flat structure is the first such example in ASC analogues.

Experimental

dASC and dASC2 analogues were synthesized by a previously reported method (Hamada *et al.*, 1987). Dimerization of dASC was controlled by the concentration of starting linear peptides. The

cyclization reaction was performed in 10 m*M* of the linear peptide, but dimerization mainly occurred above this concentration. dASC2 analogues (*ca* 20 mg) were dissolved in DMF (0.2 ml), and water was added to the solution just before the solution became unclear. The aqueous DMF solution was sealed in vials at room temperature. A crystal was mounted on a nylon loop in the mother liquor, and frozen under a nitrogen stream (100 K).

Crystal data

$C_{72}H_{112}N_{16}O_{16}S_4\cdot3C_3H_7NO\cdot H_2O$
M_r = 1823.32
Monoclinic, $P2_1$
a = 11.5563 (1) Å
b = 9.9957 (2) Å
c = 42.9208 (10) Å
β = 91.3492 (15)°
V = 4956.55 (16) Å^3
Z = 2
D_x = 1.222 Mg m^{-3}
Synchrotron radiation
λ = 0.83600 Å
Cell parameters from 3722 reflections
θ = 0.8–10.0°
μ = 0.17 mm^{-1}
T = 100 (2) K
Cubic, colourless
0.01 × 0.01 × 0.01 mm

Data collection

Rigaku RAXIS–IV diffractometer
Oscillation scans
Absorption correction: cylindrical (*CYLABS*; Nardelli, 1998)
T_{min} = 0.657, T_{max} = 0.661
15 629 measured reflections
8977 independent reflections
8890 reflections with $I > 2\sigma(I)$
R_{int} = 0.038
θ_{max} = 30.3°
$h = -13 \rightarrow 13$
$k = -11 \rightarrow 12$
$l = 0 \rightarrow 51$

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.097
$wR(F^2)$ = 0.259
S = 1.25
8977 reflections
1119 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.1312P)^2 + 5.0564P]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.017
$\Delta\rho_{max}$ = 1.52 e Å^{-3}
$\Delta\rho_{min}$ = −1.21 e Å^{-3}
Absolute structure: Flack (1983)
Flack parameter = −0.17 (14)

Table 1
Hydrogen-bonding geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
N9—H12···O12	0.86	2.57	2.779 (7)	95
N16—H19···O12	0.86	2.64	2.957 (6)	103
C24—H28···O96	0.93	2.60	3.158 (11)	119
N36—H40···O81	0.86	2.44	3.240 (9)	155
O39—H43···O119	0.84	1.78	2.58 (2)	157
N63—H68···O66	0.86	2.55	2.784 (7)	97
N70—H75···O66	0.86	2.76	2.942 (7)	94
N70—H75···O114	0.86	2.15	2.963 (13)	157
C78—H84···O42	0.93	2.29	3.190 (12)	164
N90—H96···O27	0.86	2.13	2.952 (10)	161
O93—H99···O124	0.85	1.88	2.72 (2)	172
O124—H135···O81	0.85	1.95	2.80 (2)	174
N16—H19···O109^{i}	0.86	2.12	2.965 (9)	167
O12—H15···O8^{ii}	0.82	1.91	2.712 (6)	167
N9—H12···O15^{ii}	0.86	2.10	2.873 (6)	150
N43—H47···O89^{iii}	0.86	2.02	2.862 (8)	166
O66—H71···O62^{iv}	0.82	1.86	2.684 (7)	179
N63—H68···O69^{iv}	0.86	2.02	2.823 (7)	154
N97—H103···O35^{v}	0.86	2.31	3.059 (9)	146

Symmetry codes: (i) $x, 1+y, z$; (ii) $-x, \frac{1}{2}+y, -z$; (iii) $x-1, y, z$; (iv) $-x, \frac{1}{2}+y, 1-z$; (v) $1+x, y, z$.

Table 2
Selected torsion angles (°).

C107—N1—C2—C7	φ1	−128.0 (6)
N1—C2—C7—N9	ψ1	158.6 (5)
C2—C7—N9—C10	ω1	177.0 (5)
C7—N9—C10—C14	φ2	54.2 (7)
N9—C10—C14—N16	ψ2	43.0 (6)
C10—C14—N16—C17	ω2	174.9 (5)
C14—N16—C17—C21	φ3	120.2 (5)
N16—C17—C21—N22	ψ3	169.3 (5)
C17—C21—N22—C23	ω3	172.1 (6)
C21—N22—C23—C26	φ4	−179.7 (7)
N22—C23—C26—N28	ψ4	−4.0 (12)
C23—C26—N28—C29	ω4	−172.4 (8)
C26—N28—C29—C34	φ5	−138.5 (9)
N28—C29—C34—N36	ψ5	138.3 (9)
C29—C34—N36—C37	ω5	167.3 (8)
C34—N36—C37—C41	φ6	−138.6 (9)
N36—C37—C41—N43	ψ6	132.2 (8)
C37—C41—N43—C44	ω6	−177.8 (7)
C41—N43—C44—C48	φ7	95.4 (8)
N43—C44—C48—N49	ψ7	−154.2 (7)
C44—C48—N49—C50	ω7	179.6 (7)
C48—N49—C50—C53	φ8	−178.1 (7)
N49—C50—C53—N55	ψ8	−4.8 (10)
C50—C53—N55—C56	ω8	171.7 (6)
C53—N55—C56—C61	φ9	−129.8 (7)
N55—C56—C61—N63	ψ9	151.6 (5)
C56—C61—N63—C64	ω9	171.2 (5)
C61—N63—C64—C68	φ10	63.9 (7)
N63—C64—C68—N70	ψ10	32.5 (7)
C64—C68—N70—C71	ω10	173.2 (5)
C68—N70—C71—C75	φ11	120.5 (6)
N70—C71—C75—N76	ψ11	157.4 (6)
C71—C75—N76—C77	ω11	174.1 (7)
C75—N76—C77—C80	φ12	178.8 (8)
N76—C77—C80—N82	ψ12	−2.8 (12)
C77—C80—N82—C83	ω12	−170.0 (8)
C80—N82—C83—C88	φ13	−102.1 (10)
N82—C83—C88—N90	ψ13	132.2 (8)
C83—C88—N90—C91	ω13	170.9 (8)
C88—N90—C91—C95	φ14	−134.9 (8)
N90—C91—C95—N97	ψ14	125.0 (8)
C91—C95—N97—C98	ω14	174.2 (7)
C95—N97—C98—C102	φ15	84.8 (8)
N97—C98—C102—N103	ψ15	−146.4 (7)
C98—C102—N103—C104	ω15	179.7 (6)
C102—N103—C104—C107	φ16	−178.2 (6)
N103—C104—C107—N1	ψ16	−7.4 (10)
C2—N1—C107—C104	ω16	171.0 (6)

A total of 90 images with oscillation-angle of 2° were measured on a synchrotron using RAXIS-IV, and the first 87 frames were processed by *MOSFLM* (Leslie, 1999). The 37199 observed reflections were successively processed by the CCP4 program suite (CCP4, 1994), and the reflections were merged to 17325 reflections with an R_{merg} value of 0.060. The structure was solved using all reflections. H atoms of the peptide were positioned at calculated positions and constrained during the refinement. H atoms of the water molecules were positioned by considering the hydrogen-bonding networks and fixed during the refinement. The resolution of the diffraction data was 0.80 Å, but reflections of 0.83–0.80 Å resolution were omitted in the final refinement stage because of the higher R value of that shell. Disorder was observed in the D-Val7 side chain, relating to the rotation of the Cα—Cβ bond. The occupancies of the two disordered sites were 0.59 and 0.41.

Data collection: *PROCESS* (Higashi, 1996); cell refinement: *MOSFLM* (Leslie, 1999); data reduction: *MOSFLM*; program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular

graphics: *PLATON* (Spek, 1998); software used to prepare material for publication: *PARST* (Nardelli, 1995).

The beam time of SPring-8/BL24 for this study was provided from Hyogo Prefecture and Japan Synchrotron Radiation Research Institute (Approval No. C00A24XU-5003N).

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