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A flat squared conformation of an ascidiacyclamide derivative caused by chiral modification of an oxazoline residue

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Abstract

We designed a deoxazoline-ascidiacyclamide (*d*ASC), cyclo(–L–Ile–L–*allo*–Thr–D–Val–thiazole–)$_2$, diastereomer having 10*S*, 11*R*, 37*R*, and 38*S* configurations ([*SR,RS*]*d*ASC) and a corresponding product having 10*S*, 11*S*, 37*R*, and 38*R* configurations ([*SS,RR*]ASC) with the aim of understanding better the relationship between conformational behaviour and chirality. X-ray diffraction analysis revealed that [*SR,RS*]*d*ASC is folded in a manner similar to other *d*ASC analogues. By contrast, [*SS,RR*]ASC is a novel, flat conformer that is larger than the major square and folded ASC conformers and contains a cavity created by the flat peptide ring. In addition, [*SS,RR*]ASC retains approximately 60% of the cytotoxicity of the parent molecule.

Keywords: Ascidiacyclamide; Deoxazoline-ascidiacyclamide; Diastereomer; Oxazoline ring; X-ray structure; Cytotoxicity

Ascidiacyclamide (ASC), cyclo(–L–Ile–L–Oxz–D–Val–Thz–)$_2$, is a cytotoxic cyclic peptide isolated from tunicates that contain the unusual amino acids, thiazole (Thz) and oxazoline (Oxz) [1]. The chemical structure of ASC is characterized by a C_2-symmetric sequence (Fig. 1), whose NMR spectroscopic and X-ray diffraction analyses have shown to exist in major conformations: square and folded [2–7]. To better understand the conformational behaviour of ASC, we previously carried out asymmetric modifications in which the Ile[1] residue was replaced with another amino acid (Gly, Ala, Aib, Leu, Val, or Phe) to disturb the C_2-symmetry [8,9]. These modifications showed that the conformational equilibrium between the square and folded forms is related to the bulkiness of the side chain of the substituting amino acid [9]. In that regard, Oxz residues, whose rings are located at the pivot of the folded structure, are conformationally restricting. Removing this residue thus increases the conformational flexibility of the ASC molecule, enabling compact folding. It is not surprising therefore that X-ray diffraction analysis showed the structures of all deoxazoline-ascidiacyclamide (*d*ASC) analogues to be folded [10–12].

Furthermore, both *d*ASC and ASC analogues form complexes with copper, zinc, calcium, and potassium ions [13–16]. However, the structures of the *d*ASC-ion complexes differ from the major ASC forms [13,14], suggesting that the "rigid block" moieties, which include Oxz and Thz, limit the conformation of ASC to only two conformers.

It thus appears that the Oxz residue is a strong determinant of ASC molecular folding. Our focus has been on the chirality of the second and sixth residues of *d*ASC and ASC. Thr and Oxz residues each have chiral carbons at the α and β positions. As shown in Fig. 1, the Oxz rings in ASC are formed from *d*ASC by reaction with thionyl chloride [17–20], which reverses the chirality of β carbons. Therefore, ASC having 10*S*, 11*R*, 37*S*, and 38*R* configurations is synthesized from *d*ASC having 10*S*, 11*S*, 37*S*, and 38*S* configurations. In this report, we describe our design of a *d*ASC diastereomer having 10*S*, 11*R*, 37*R*, and 38*S* configurations ([*SR,RS*]*d*ASC) and its product, an ASC analogue

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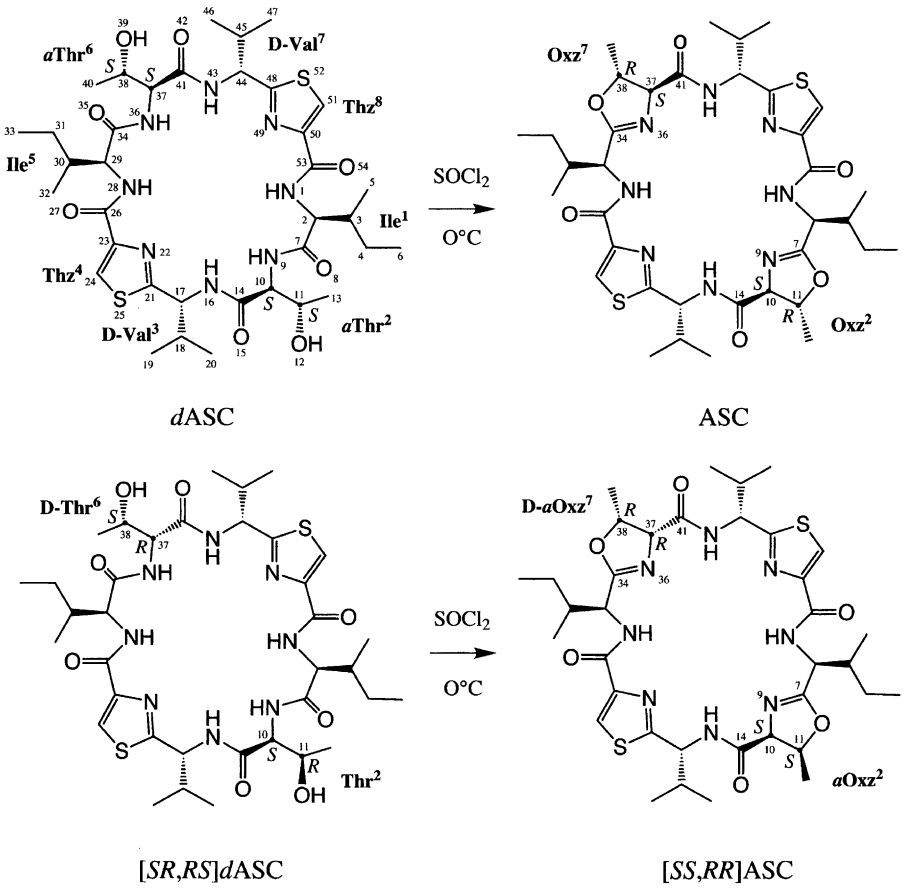

Fig. 1. Chemical structures of *d*ASC, ASC, [*SR,RS*]*d*ASC, and [*SS,RR*]ASC.

having 10*S*, 11*S*, 37*R*, and 38*R* configurations ([*SS,RR*]ASC). Their structures were determined by X-ray diffraction.

Materials and methods

Peptides. The synthesis of [*SR,RS*]*d*ASC and [*SS,RR*]ASC was carried out, as previously described [17–20]. For the synthesis of [*SR,RS*]*d*ASC, Thr and D-Thr were incorporated at the second and sixth positions, respectively (Fig. 1); [*SS,RR*]ASC was then obtained from [*SR,RS*]*d*ASC by treatment of thionyl chloride.

X-ray diffraction. Crystals of [*SR,RS*]*d*ASC were grown from dimethylformamide solution and data collection was performed with a Rigaku AFC5R using Cu–Kα radiation. The crystal data for [*SR,RS*]*d*ASC are as follows: formula = $C_{18}H_{28}N_4O_4S$, $M_r = 396.51$, orthorhombic, $P22_12_1$, $a = 10.999(9)$ Å, $b = 19.032(9)$ Å, $c = 10.445(5)$ Å, $V = 2187(2)$ Å^3, $Z = 2$, $D_x = 1.204\,g\,cm^{-3}$, $F(0\,0\,0) = 848$, μ(Cu–Kα) $= 1.558\,mm^{-1}$, absorption corrections by ψ-scan, $T_{min} = 0.76$, $T_{max} = 0.91$, number of measured reflections = 4223, $\theta_{max} = 63.98°$, number of total reflections = 3382, $R_{int} = 0.0573$, number of reflections with $> 2\sigma(I) = 2640$, Flack × parameter = −0.08(8), number of parameters = 281, $R = 0.0996$, w$R = 0.2447$, goodness of fit = 1.074, $(\Delta/\sigma)_{max} = 0.004$, $\Delta\rho_{max} = 0.564\,e$Å^{-3}, and $\Delta\rho_{min} = -0.238\,e$Å^{-3}. The structure was solved using SHELXS-97 [21] and refined with SHELXL-97 [22]. Hydrogen atoms were positioned at the calculated positions and constrained during the refinement. The twofold axis was located on the molecule and the crystallographically independent atoms were a half content of the chemical formula. The threonine residue was disordered at two sites within the crystal that correspond to Thr and D-Thr, respectively. The structure determination was attempted for the space group $P2_1$, but disordering at both threonine residues was observed. This means that the [*SR,RS*]*d*ASC molecule rotates within the crystal, despite the asymmetric chemical structure. The whole [*SR,RS*]*d*ASC molecule is drawn in Fig. 2, with the disordered threonine residues separated and drawn at the second and sixth positions.

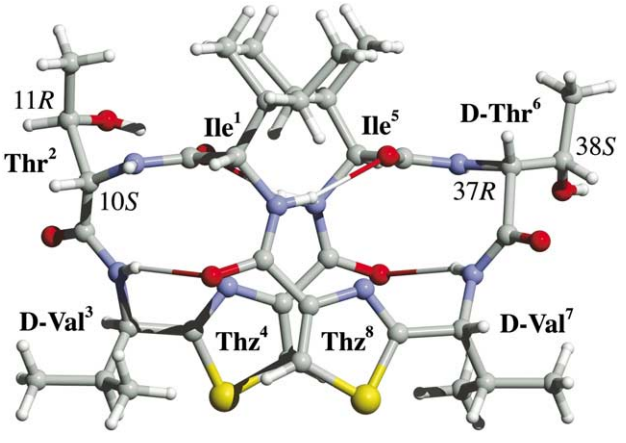

Fig. 2. Stereo view of the structure of [*SR,RS*]*d*ASC. The crystallographic twofold axis is located on the molecule, but the whole molecule is drawn to show the entire structure. The disordered threonine residues are separated for drawing purposes. Thin lines represent hydrogen bonds. Figures were drawn by RasMol [28] and POV-Ray [29].

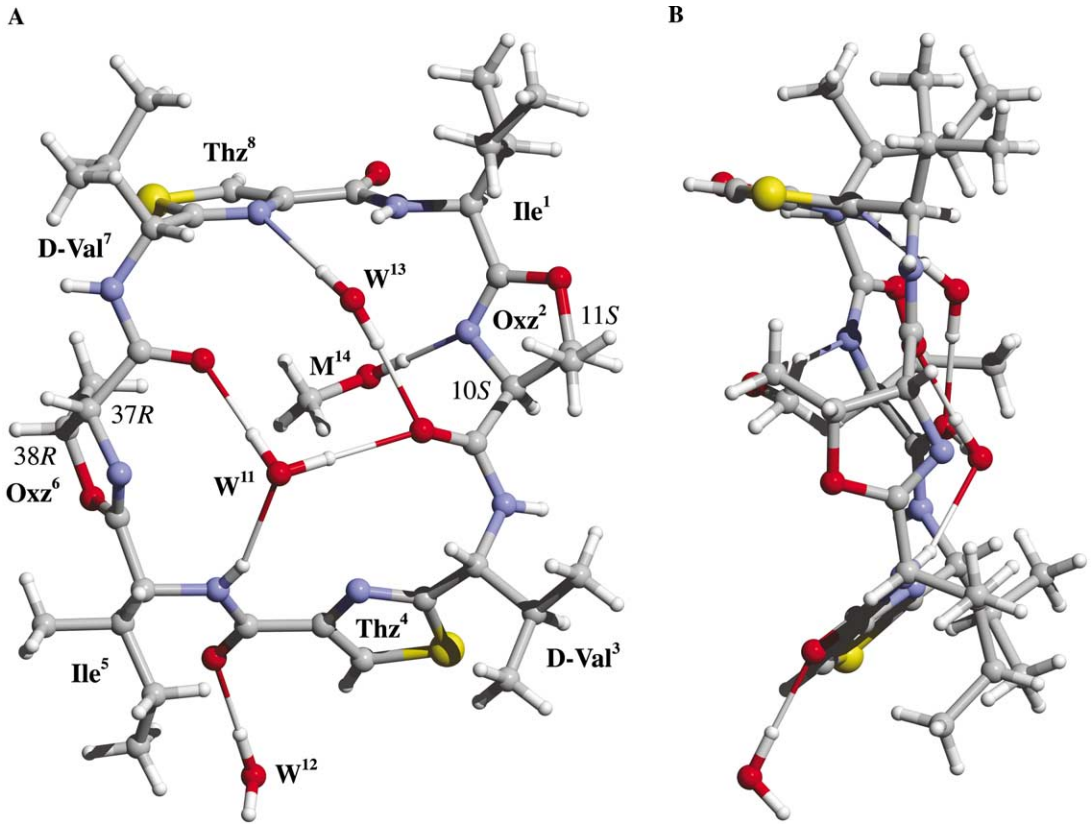

Fig. 3. Stereo views of the structure of [*SS,RR*]ASC. Shown are top (A) and side (B) views of the peptide ring. "W" and "M" represent the water and methanol molecules, respectively; thin lines represent hydrogen bonds. Figures were drawn by RasMol [28] and POV-Ray [29].

Crystals of [*SS,RR*]ASC were grown from aqueous methanol solution and data collection was performed at a synchrotron (SPring-8 BL24XU-A) using a Rigaku R-AXIS IV. Crystals were briefly passed through 40% glycerol and flash-frozen in a nitrogen stream (100 K). Collected image data were integrated using MOSFLM [23] and CCP4 suit [24]. The crystal data for [*SR,RS*]*d*ASC are as follows: formula = $C_{36}H_{52}N_8O_6S_2 \cdot 3(H_2O) \cdot CH_3OH$, $M_r = 843.07$, monoclinic, $P2_1$, $a = 14.4745(4)$ Å, $b = 9.9580(2)$ Å, $c = 15.3414(4)$ Å, $\beta = 94.859(2)°$, $V = 2203.32(9)$ Å^3, $Z = 2$, $T = 100(2)$ K, $D_x = 1.271$, $F(000) = 904$, wavelength = 0.836 Å, $\mu = 0.182\,mm^{-1}$, number of measured reflections = 4543, $\theta_{max} = 31.35°$, number of reflections with $> 2\sigma(I) = 4423$, Flack × parameter = 0.02(12), number of parameters = 515, $R = 0.0536$, w$R = 0.1387$, goodness of fit = 1.136, $(\Delta/\sigma)^{max} < 0.001$, $\Delta\rho_{max} = 0.555\,e\,Å^{-3}$, and $\Delta\rho_{min} = -0.627\,e\,Å^{-3}$. The structure of [*SS,RR*]ASC was solved using SHELXS-97 [21] and refined with SHELXL-97 [22]. The hydrogen atoms of the peptide were positioned at the calculated positions and constrained during the refinement; the water molecules were positioned by considering hydrogen-bonding networks and fixed during the refinement. The structure of [*SS,RR*]ASC is shown in Fig. 3.

Cytotoxicity measurement. Cytotoxicity of [*SR,RS*]ASC was evaluated in P388 lymphocytic leukemia cells using the previously described method with some modification [25,26].

Results and discussion

The [*SR,RS*]*d*ASC molecule is folded so that the Thz rings are facing one another (Fig. 2) and the folded backbone is stabilized by four intramolecular hydrogen bonds (Ile^1 NH···,Ile^5 O, D-Val^{3}NH···O Thz^8, Ile^{5}NH···Ile^{1}O, and D-Val^7 NH···Thz^{4}O). The difference in Cα chirality resulted in a difference in the conformations of the Thr^2 and D-Thr^6 side chains: the Cα–Cβ bond is parallel to the bond stream of Ile^1 Cα–Ile^1 C–Oxz^2 N–Oxz^2 Cα at the Thr^2 residue, as well as to that of Thr^6 Cα–Thr^6 C–D-Val^7 N–D-Val^7 Cα at the D-Thr^6 residue. Still, the manner in which [*SR,RS*]*d*ASC folds is similar to the way other known forms fold [10–12], which indicates that switching the Cα chirality of one of the two Thr residues (the 37*R* configuration in this case) has little effect on the molecular folding of the *d*ASC analogue.

By contrast, the structure of [*SS,RR*]ASC is unique (Fig. 3); for comparison, the square form of ASC is shown in Fig. 4. In the square form, the Oxz and Thz rings are located at the corners of the square and the peptide backbone is bent (Fig. 4A). In the present form, Ile and D-Val residues are located at the corners of the square (Fig. 3A) and the peptide ring is relatively flat (Fig. 3B). Conformational differences are also found in the torsion angles (Table 1). The $\psi 1$, $\psi 2$, $\psi 3$, $\psi 5$, $\psi 6$, and $\psi 7$ angles of the folded and square forms are quite different from [*SS,RR*]ASC, whose relatively flat ring creates a cavity in the molecule. Moreover, the Oxz^{2}N···Oxz^{6}N and Thz^{4}N···Thz^{8}N distances within the flat form of [*SS,RR*]ASC (7.65 and 7.66 Å, respec-

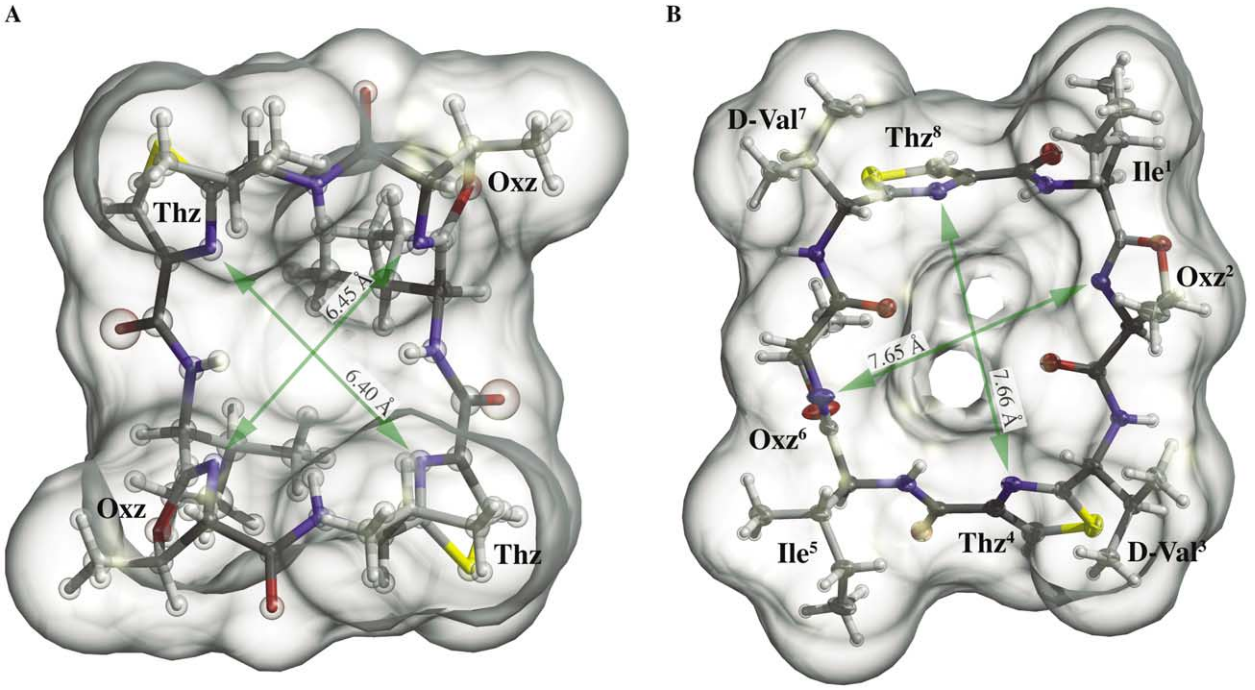

Fig. 4. Molecular structures of (A) the square form of ASC and (B) the flat square form of [*SS,RR*]ASC with the accessible surfaces. Green arrows represent the Oxz^2 N···Oxz^6N and Thz^4 N···Thz^8 N distances. ASC was cocrystallized with benzene molecules [2,3]; they were omitted for clarity. The accessible surfaces were calculated by MSMS [27] and figures were drawn by Raster3D [30].

Table 1
Torsion angles (°) of the peptide backbones of *d*ASC and ASC analogues

Angle	Bond number	[*SR,RS*]*d*ASC a[a]		[*SR,RS*]*d*ASC b[a]		
	[*SS,RR*]ASC	Fold[b]	Square[c]			
ϕ1	53-1-2-7	−72.4(8)		−122.1(5)	−76	−139.1
ψ1	1-2-7-9	120.6(7)		36.4(7)	114.6	−25.4
ω1	2-7-9-10	177.7(17)	−161.9(14)	174.0(5)	−175.1	−172.7
ϕ2	7-9-10-14	72.3(21)	62.2(30)	133.3(5)	100.7	115.8
ψ2	9-10-14-16	14.7(19)	18.5(28)	155.4(4)	−4.2	28.1
ω2	10-14-16-17	−171.5(13)	−167.9(15)	179.8(4)	−162.1	−174.5
ϕ3	14-16-17-21	117.6(6)		86.8(6)	100.8	121.8
ψ3	16-17-21-22	−24.3(11)		−100.8(6)	−28.1	58.6
ω3	17-21-22-23	−175.1(8)		177.4(5)	−172.6	178.6
ϕ4	21-22-23-26	−179.6(7)		177.2(5)	−179.8	−177.6
ψ4	22-23-26-28	−1.5(11)		2.4(7)	−0.5	−11.8
ω4	23-26-28-29	−177.7(7)		−175.1(5)	−175.1	−179.4
ϕ5	26-28-29-34			−122.7(5)	−82.2	−143.5
ψ5	28-29-34-36			−81.0(7)	112.4	−24.8
ω5	29-34-36-37			180.0(5)	−172.3	−174.3
ϕ6	34-36-37-41			−121.2(5)	105.7	116.3
ψ6	36-37-41-43			−151.8(5)	−1.7	30.4
ω6	37-41-43-44			179.9(4)	−163.1	−174.4
ϕ7	41-43-44-48			65.7(6)	90.1	117.5
ψ7	43-44-48-49			−120.4(6)	−16.2	58.3
ω7	44-48-49-50			−178.3(5)	−176.4	177.7
ϕ8	48-49-50-53			178.7(5)	178.6	−176.9
ψ8	49-50-53-1			5.9(7)	−2.8	−11.3
ω8	50-53-1-2			177.4(4)	−174.2	167.2

[a] Half molecule of the chemical structure was crystallographically independent and the Thr residue was disordered to two sites.
[b] Torsion angles were cited from the crystal structure of [Ala^1]ASC [9].
[c] Torsion angles cited from the crystal structures of [Val^1]ASC [9] were averaged.

tively) are longer than those in the square form (6.45 and 6.40 Å, respectively). The accessible surface drawings calculated by MSMS [27] indicate the cavity created in the flat form, but no space in the square form (Fig. 4). Two water molecules (W^{11} and W^{13}) and one methanol molecule (M^{14}) are situated inside of the peptide (Fig. 3). The W^{11} molecule interacts with Oxz^2 O, Oxz^6O, and Ile^5 NH, while the W^{13} molecule interacts with Oxz^2 O and Thz^8 N; the M^{14} molecule interacts with Oxz^2 N. The crystal of the square form of ASC contains some solvent molecules, but their positions are not inside the ASC molecule [2,3,6,7].

Earlier studies have suggested that the cytotoxicity of ASC is related to its structure [5,6]. In studies of asymmetric modification, spectroscopic and X-ray diffraction analyses showed that ASC analogues incorporating aliphatic amino acids of appropriate size are strongly cytotoxic and that such analogues are in the square from. When the cytotoxicity of [*SS,RR*]ASC was evaluated using P388 lymphocytic leukaemia cells, we obtained an IC_{50} value of 17.5 μg/ml, which is approximately 60% that of the parent molecule ($IC_{50} = 10.5$ μg/ml) and in the same range as the ASC epimers cyclo(-D-Ile–Oxz–D-Val–Thz–Ile–Oxz–D-Val–Thz–) and cyclo(–Ile–Oxz–Val–Thz–Ile–Oxz–D-Val–Thz–) [8], which showed unique behaviour in various NMR titrations. We do not have clear evidence, but chiral modifications may result in conformational changes that conserve cytotoxicity. The present flat-square form differs from the square form, but is more like it than the folded form. We suggest that certain characteristics common to the square and flat-square forms are key determinants of ASC activity.

The present "flat-square" form of [*SS,RR*]ASC is the first example of a crystal structure of ASC or its derivatives shown to differ from the two major ASC forms. Modification of the first residue of ASC determines to some extent whether the molecule assumes the square or folded form; chiral modification for the Oxz residues results in a novel structure and is indicative of the key role played by Oxz residues in determining ASC conformation.

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