Scientific paper

An Axial Chirality and Disorder of Positional Isomers in a Crystal of Highly Cytotoxic 3-acetyl-1,3-*bis* (2-chloro-4-nitrophenyl)-1*E*-triazene

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Dedicated to the memory of Prof. Dr. Jurij V. Brenčič.

Abstract

Biologically active 4-nitro-substituted 1,3-diaryltriazene, a chemical analogue of 1,3-*bis*(4'-amidinophenyl)-triazene-berenil®, belongs to the novel, chemically modified class of potent antitumor agents. Its structural characterization by X-ray analysis and ^{1}H NMR spectroscopy is performed to determine molecular overall conformation in view of its possible interaction to DNA.

Keywords: Axial chirality, Enantiomeric disorder, Molecular structure, Crystal structure

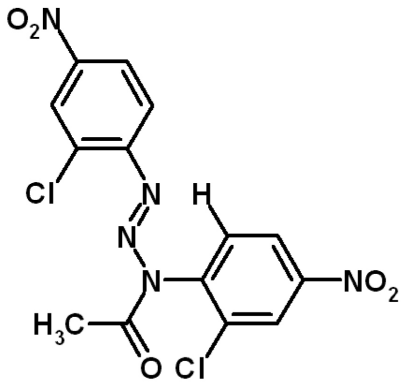
1. Introduction

The synthesis and biological activity of a new class of 4-nitro-substituted 1,3-diaryltriazenes have revealed that this class can be modified from inactive to highly cytotoxic compounds by substitution of the two nitro groups at the *para* position of benzene rings and two additional electron withdrawing groups at the *ortho* position.[1,2] To increase solubility of these compounds the triazene nitrogen was acetylated, however, LC-MS/MS analysis pointed out that *N*-acyltriazenes are prodrugs of non-acylated triazenes. This class of compounds exhibit anticancer activity. The selected representative of this group, 3-acetyl-1,3-*bis*(2-chloro-4-nitrophenyl)-1*E*-triazene preferentially targets malignant cells. Since its antiproliferative activity is significantly higher against tumor cells than against normal cells, the above mentioned compound may serve as a potential antitumor agent.[1] DNA binding analysis suggests that 3-acetyl-1,3-*bis*(2-chloro-4-nitrophenyl)-1*E*-triazene and its non-acylated derivative do not bind into the minor groove of DNA.[1] Instead, it induces reactive oxygen species and the endoplasmatic stress response, as well as independent activation of stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK) pathway.[1,3]

Various triazenes have been synthesized and thoroughly investigated over a few decades due to their potential in human and veterinary medicine.[4 and references therein] However, their mechanisms of activity at molecular level have not been clarified yet. Among all triazenes, binding mode of berenil, 1,3-*bis*(4'-amidinophenyl)-triazene, has been examined experimentally and by molecular modelling that proved heterogeneous DNA-binding modes.[5] However, the generally accepted mode of berenil binding is *via* complexation into the minor-groove of the A-T reach domains of DNA double helices and also RNA.[6,7] Some other stereochemically feasible berenil-DNA complexes can be modelled with the drug partially intercalated through the minor groove or pseudo-intercalated through the major groove.[5] Both models revealed the global helical parameters of DNA similar to the same DNA in the absence of the drug and in the original crystal structure of the decamer.[5,8] In addition to this classic duplex minor grove binding, G-quadruplex binding was reported, recently; [9] the experiments also revealed that berenil is not as selective for AT-rich duplexes as previously thought. Its inhibiting activity of topoisomerases was established as well.[10,11]

Therefore, among of all 4-nitro-substituted 1,3-diaryltriazenes synthesized, we selected 3-acetyl-1,3-

bis(2-chloro-4-nitrophenyl)-1E-triazene (Scheme 1), as the most cytotoxic against different tumor cell lines,[1] to determine its molecular structure and overall conformation in the solid state by X-ray structure analysis and ^{1}H NMR spectroscopy. Its molecular structure should provide structural evidences why the title compound is not suitable for an intercalation into DNA.



Scheme 1

2. Results and Discussion

The molecular graph (Scheme 1) indicates that the title molecule cannot be planar due to a steric hindrance and its crystal structure reveals strongly non-planar mo-

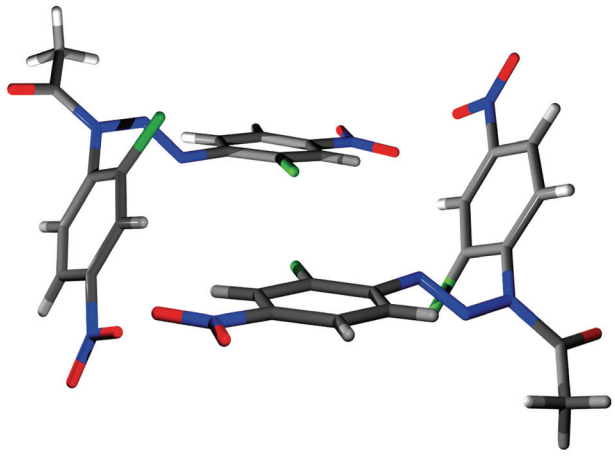


Figure 1. An asymmetric unit with the two molecules **A** and **B** related by a pseudo-inversion centre.

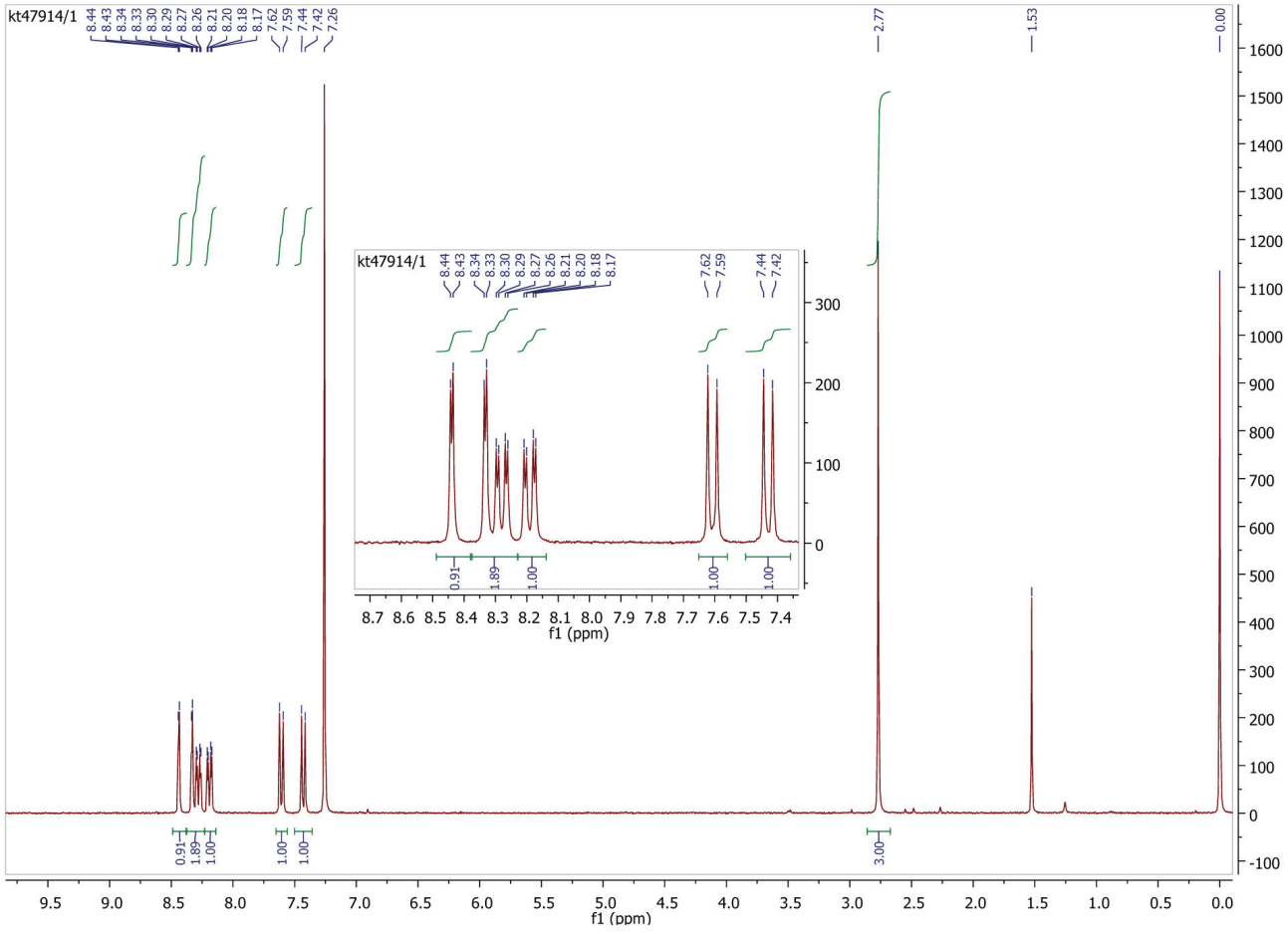
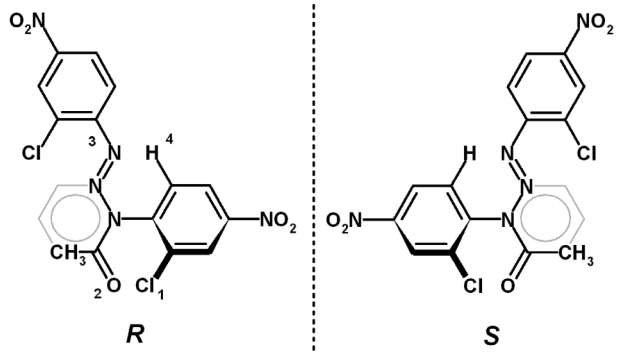


Figure 2. ^{1}H NMR spectrum of 3-acetyl-1,3-*bis*(2-chloro-4-nitrophenyl)-1*E*-triazene in $CDCl_3$ solution.

Table 1. Torsional angles defining enantiomeric relations of *R*-**A** and *S*-**B** molecules.

	***S*-A**	***R*-A**	***R*-B**	***S*-B**
·Cl1/H3–C3–C4–N2	–5.70 (12)	5.70 (12)	4.27 (12)	–4.27 (12)
·Cl2/H5–C5–C4–N2	–11.9 (16)	11.9 (16)	–3.2 (12)	3.2 (12)
C3–C4–N2–N3	–75.6 (14)	75.6 (14)	–78.4 (12)	78.4 (12)
C5–C4–N2–N3	103.1 (13)	–103.1 (13)	100.5 (11)	–100.5 (11)
C4–N2–N3–N4	6.1 (13)	–6.1 (13)	3.8 (12)	–3.8 (12)
N2–N3–N4–C7	–177.7 (7)	177.7 (7)	–178.7 (7)	178.7 (7)
N3–N4–C7–C8	147.6 (8)	–147.6 (8)	141.8 (8)	–141.8 (8)

·Two different orientations of the ring (resulting in atropisomers), require the atom sequences to start with different atoms.



Scheme 2. Chirality of the title compound can be described in analogy to substituted biphenyls. The planar moiety N=N-N-CO-CH_3 can be regarded as a fragment of a substituted phenyl ring (indicated in gray). Due to a hindered rotation, midpoints of N2–C4(phenyl) bonds can be regarded as a stereogenic centre; four different substituents are -H, -Cl, =N and =O and their priority is indicated defining the absolute configuration of axially chiral molecule. The numbers assigned to atoms assign the priority important for defining the absolute configuration according to CIP rules.[13–15]

lecular conformation. The dihedral angles between two planes of the aryl groups in both conformers characterize puckered molecules [67.2(5) in molecule **A** and 62.3(5)° in molecule **B**]. Two symmetry-independent molecules (**A** and **B** in the asymmetric unit) (Fig. 1) are related by a pseudo-inversion centre located approximately at (0.75, 0.12, 0.12). Conformations of **A** and **B** are nearly identical within experimental error (the differences in torsional angles are about 3 uncertainty units, Table 1). Due to the presence of the pseudo-inversion centre, **A** and **B** molecules are almost in an enantiomeric relationship.

A hindered intramolecular rotation around N-C_{phenyl} bond leads to a disorder of the phenyl ring vicinal to the acetyl group in order to optimize the crystal packing. The chlorine atom is disordered over two positions, so that a half of a chlorine atom is bound to each α-C atom of the ring. Therefore, the two isomers are present, occupying the same crystallographic position (both occupancies are 50%). However, they could not be distinguished by NMR spectra, as no splitting of the signals was detected (Fig. 2, Section 4.1). The only explanation can be that the molecule is chiral: the two disordered isomers observed in the crystal structure are actually enantiomers and the crystals are racemic.

Chiral molecules lacking asymmetrically substituted atom(s) are usually referred to as axially chiral or helical.[12–14] In many compounds similar to biphenyls intramolecular rotation which would transform a left- into a right-handed conformer is hindered due to sterically bulky substituents. Such conformers which may be stable at room temperature are referred to as atropisomers. In some cases, such as chiral biphenyls and allenes, a stereogenic centre (not necessarily an atom) may be defined and an absolute configuration may be designated according to CIP-rules.[13–15] The »stereogenic centre« is located at the midpoint of the central C–C bond, and *R*/*S* configuration is determined by positions of the four substituents, analogous to the configurations of the tetrahedral carbon atom. Due to non-planarity of the title compound and hindered intramolecular rotation, such a centre can be recognised at the midpoint of C4–N2 bond (Scheme 1, Fig. 3). Due to sterical reasons, rotation around the bond is hindered. The phenyl ring and the N=N-N-CO-CH_3 moiety are planar and conformationally rigid; therefore the N=N-N-CO-CH_3 moiety can also be regarded as a fragment of the phenyl ring (Scheme 2). Four different substituents are identified around the C4-N2 bond: H and Cl are bound to the phenyl, while =N and =O are bound to the NNC »phenyl fragment« (Scheme 2). Priorities are assigned to these substituents according to the CIP-rules. Therefore, *R* and *S* configurations can be assigned to different conformations of the molecules **A** and **B** according to the CIP-rules (Scheme 2).

Generally, any object (molecule) in a 3D space is chiral if it comprises four non-equivalent non-coplanar points (atoms). The four points may be linked by three non-coplanar vectors, which span either a left- or a right-handed coordinate system; alternatively, three non-coplanar vectors define a fragment of a left- or a right-handed helix. Since both *R*- and *S*-enantiomers of each molecule (**A** and **B**) are present, the four molecules can be described in the asymmetric unit (Fig. 3) and can be designated either as (*R*-**A**, *S*-**B**) or (*R*-**B**, *S*-**A**).

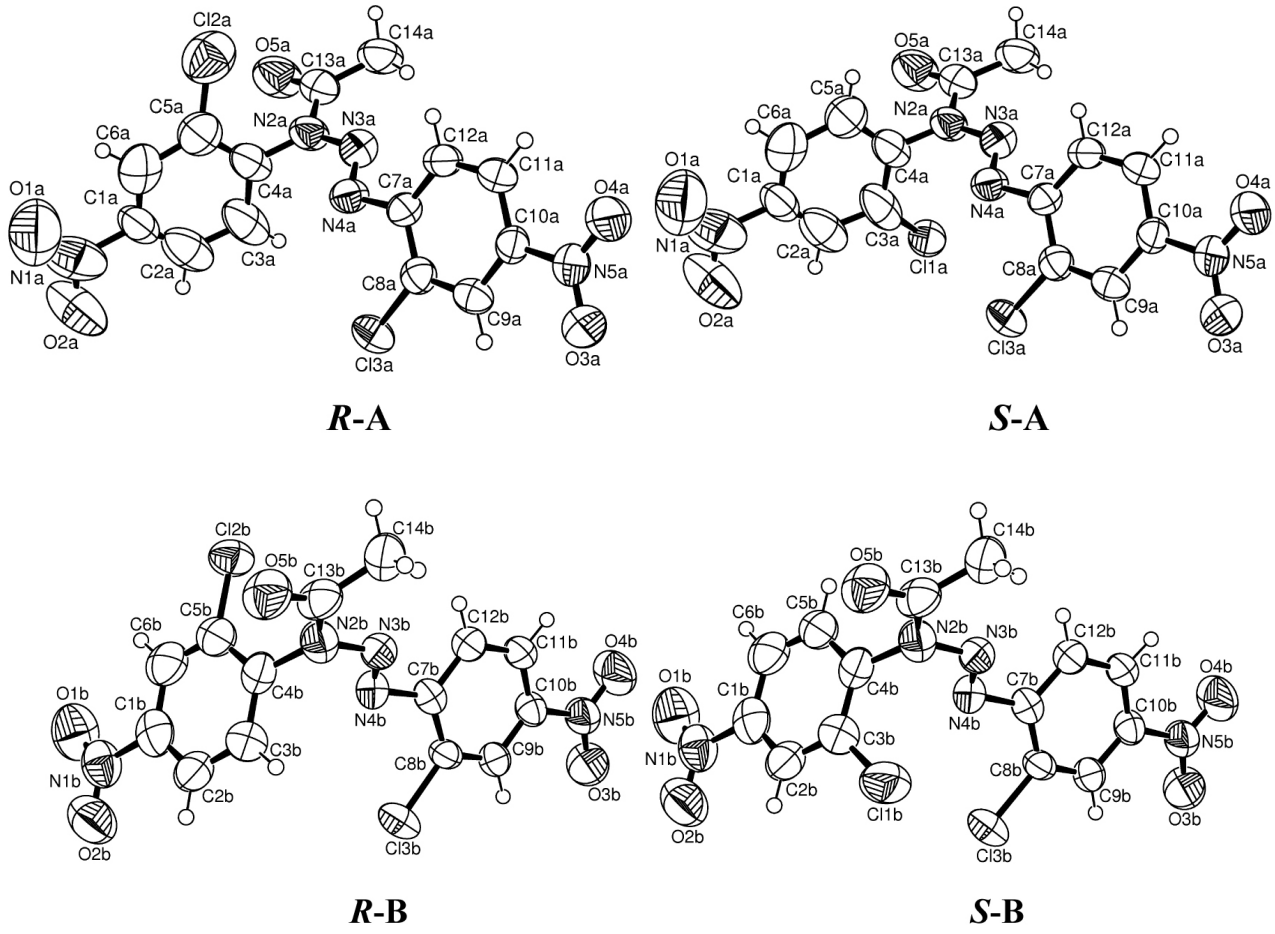


Figure 3. ORTEP-3[16] drawings of two atropisomers (left and right) of two symmetry-independent molecules (**A** and **B**). Displacement ellipsoids are drawn for the probability of 50% and hydrogen atoms are shown as spheres of arbitrary radii.

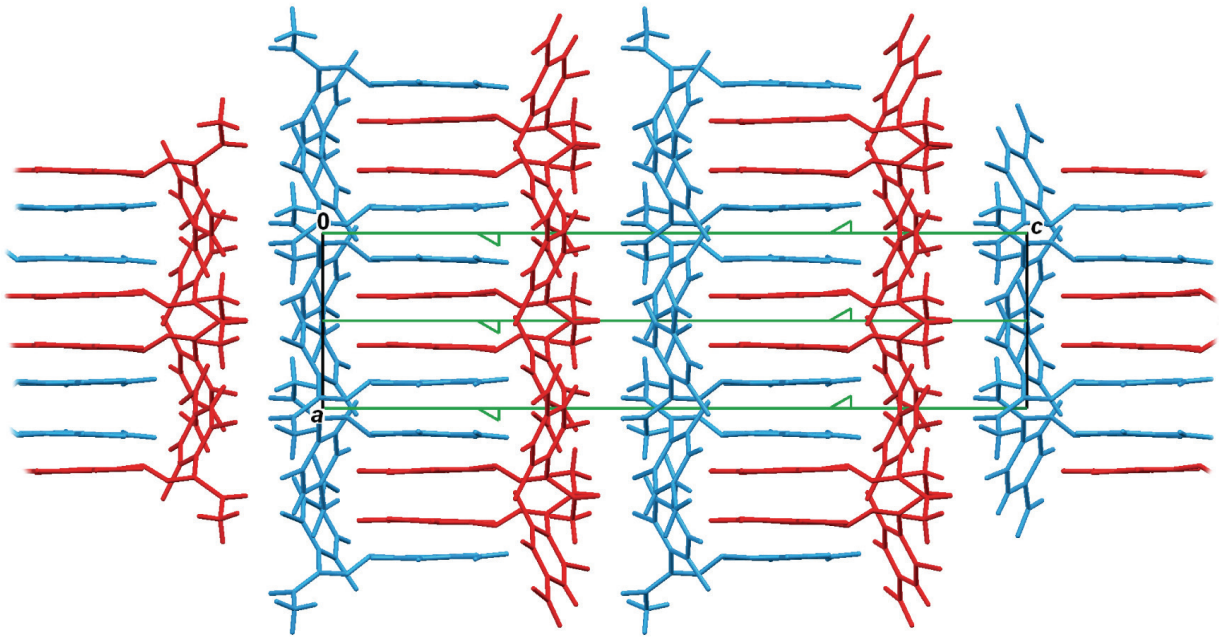


Figure 4. Crystal packing of **1** viewed down the direction [010]. For clarity, only enantiomeric pair of **A** (red) and **B** (blue) molecules are shown; hydrogen bonds have been omitted. Screw-twofold axes are shown in green.

Table 2. Geometric parameters of hydrogen bonds.

	***D*–H / Å**	**H···*A* / Å**	***D*···*A* / Å**	***D*–H···*A* / °**	**Symm. op. on *A***
C9A–H9A···O2B	0.93	2.56	3.427(15)	156	*x*, *y*, *z*
C14A–H14A···Cl2A	0.96	2.50	3.449(13)	170	–1+*x*, *y*, *z*
C14B–H14E···Cl2B	0.96	2.56	3.483(13)	162	1+*x*, *y*, *z*
C14B–H14F···Cl2A	0.96	2.82	3.724(13)	157	2–*x*, –*y*, –1/2+*z*

Table 3. Geometric parameters of the π···π interactions

π···π	**Cg[a]···Cg / Å**	**α[b]**	**β[c]**	**Cg···plane(Cg2) / Å**	**Offset / Å**	**Symm. Op.**
C7A→C12A···C7B→C12B	3.810 (5)	1.6 (4)	20.81	3.537 (4)	1.353	1–*x*, 1/2+*y*, *z*
C7B→C12B···C7A→C12A	3.792 (5)	1.6 (4)	20.54	3.576 (4)	1.328	2–*x*, 1/2+*y*, *z*

a Cg = centre of gravity of the aromatic ring.
b α = angle between planes of two aromatic rings.
c β = angle between Cg···Cg line and normal to the plane of the first aromatic ring.

The title molecule reveals no strong proton donors, thus 3D packing is achieved through C–H···O and C–H···Cl hydrogen bonds and dispersion interactions (Fig. 4). Phenyl rings C7→C12 (non-disordered ones) form π-stacks extending in the direction [100] (Tables 2 and 3).

3. Conclusion

A sterical hindrance (overcrowding effect) brings the molecule into non-planar conformation, that leads to the axial chirality. A less-common case of disorder, including the two positional isomers, occurs. The two disordered molecules were detected in the asymmetric unit (**A** and **B**) being related as two enantiomeric pairs of molecules. The significantly puckered molecule of the title compound prevents its intercalation into DNA duplex as also revealed by biological evaluation, suggesting other events to cause cell death.[1]

4. Experimental

Melting point was determined on a Kofler micro hot stage and is uncorrected. ^{1}H NMR spectrum was recorded with a Bruker Avance DPX 300 spectrometer at 29 °C and 300 MHz, using TMS as an internal standard. ^{13}C NMR spectrum was recorded on the same instrument at 75.5 MHz and is referenced against the central line of the solvent signal (DMSO-d_6 septet at δ = 39.5 ppm). IR spectrum was obtained with Bio-Rad FTS 3000MX (KBr pellet). MS spectrum was recorded with a VG-Analytical AutoSpec Q instrument. Elemental analysis (C, H, N) was performed with Perkin Elmer 2400 Series II CHNS/O Analyzer.

4. 1. Synthesis and Characterization Data

(i) *Synthesis of the starting material*: A solution of sodium nitrite (2 mmol, 0.141 g) in water (5 mL) was added to the stirring suspension of the commercially available 2-chloro-4-nitroaniline (4 mmol, 0.704 g) in hydrochloric acid (5%, 8.6 mL) over the period of 10 min at 0 °C. The reaction mixture was stirred at r.t. for 24 hours. Then, it was cooled to 0 °C, the product was filtered off and washed with water (1 mL). The crude 1,3-*bis*(2-chloro-4-nitrophenyl)-1*E*-triazene (0.576, 81% yield) was crystallized from acetone. Mp 199–200 °C; IR (KBr) 3098, 1585, 1528, 1509, 1341, 1242, 1171, 1118, 1047, 869 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (2H, d, J = 9.0 Hz), 8.22 (dd, 2H, J_1 = 9.0 Hz, J_2 = 2.4 Hz), 8.39 (2H, d, J = 2.4 Hz), 10.47 (1H, s).

(ii) *Synthesis of 3-acetyl-1,3-bis(2-chloro-4-nitrophenyl)-1E-triazene*: Acetyl chloride (282.6 mg, 3.6 mmol) was added dropwise to the suspension of 1,3-*bis*(2-chloro-4-nitrophenyl)-1*E*-triazene (712 mg, 2 mmol) and triethylamine (405 mg, 4 mmol) in acetonitrile (10 mL) at room temperature. The reaction mixture was stirred at the same temperature for 15 min and the solid (triethylammonium chloride) was filtered off. Then, the solution was evaporated to 3–4 mL and methanol (10 mL) was added. The crude product was separated by filtration (684 mg, 86% yield) and crystallized from methanol/acetone. Mp 145–147 °C; IR (KBr) 3100, 1733, 1523, 1470, 1350, 1193, 1139 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.77 (3H, s), 7.43 (1H, d, J = 8.6 Hz), 7.60 (1H, d, J = 8.6 Hz), 8.19 (1H, dd, J_1 = 8.6 Hz, J_2 = 2.5 Hz), 8.28 (1H, dd, J_1 = 8.6 Hz, J_2 = 2.5 Hz), 8.33 (1H, d, J = 2.5 Hz), 8.43 (1H, d, J = 2.5 Hz); ^{13}C NMR (75 MHz, DMSO-*d*): δ 22.2, 120.7, 123.3, 123.6, 124.7, 125.6, 130.8, 132.4, 133.1, 139.5, 147.5, 148.6, 148.9, 171.9; MS (EI) *m/z* 397 (M^+, 0.1), 184 (100), 156 (83),

110 (33), 75 (30). Anal. for $C_{14}H_9Cl_2N_5O_5$: Calcd C, 42.32; H, 2.27; N, 17.63. Found C, 42.35; H, 2.37; N, 17.34.

4. 2. Crystallography

Single crystal measurements were performed on an Enraf-Nonius CAD-4 diffractometer, using a graphite monochromated CuK_a (1.54179 Å) radiation at room temperature [293(2) K]. Three intensity control reflections measured every 120 minutes. The WinGX standard procedure was applied for data reduction.[17] No absorption correction was applied. The structure was solved using SHELXS97 [17] and refined with SHELXL97.[18] The model was refined using the full-matrix least squares refinement; all non-hydrogen atoms were refined anisotropically. Hydrogen atoms were treated as constrained entities, using the command AFIX in SHELXL97.[18] Molecular geometry calculations were performed by PLATON,[19] and molecular graphics were prepared using ORTEP-3,[16] and CCDC-Mercury.[20] Crystallographic and refinement data for the structure reported in this paper are show in Table 4.

Supplementary crystallographic data for this paper can be obtained free of charge *via* www.ccdc.cam.ac.uk /conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk). CCDC-773227 contains the supplementary crystallographic data for this paper.

Table 4. Crystallographic, data collection and structure refinement details.

Compound	**1**
Empirical formula	$C_{14}H_9Cl_2N_5O_5$
Formula wt. / g mol^{-1}	398.16
Crystal dimensions / mm	0.40 × 0.09 × 0.07
Space group	*P b c* 2_1
a / Å	7.1152 (5)
b / Å	16.3138 (7)
c / Å	28.683 (2)
α / °	90
β / °	90
γ / °	90
Z	8
V / Å^3	3329.4 (4)
D_{calc} / g cm^{-3}	1.589
μ / mm^{-1}	3.876
Θ range / °	3.08–76.32
T / K	293 (2)
Diffractometer type	Enraf-Nonius CAD-4
Range of *h*, *k*, *l*	$0 < h < 8$;
	$0 < k < 20$;
	$-36 < l < 0$
Reflections collected	3555
Independent reflections	3555
Observed reflections	
($I \geq 2\sigma$)	2010
Absorption correction	None
R_{int}	0
R (F)	0.0634
R_w (F^2)	0.2009
Goodness of fit	1.033
H atom treatment	Constrained
No. of parameters	489
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (eÅ^{-3})	0.357; –0.205
Flack parameter	0.06 (4)

5. Acknowledgements

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Povzetek

Biološko aktiven 4-nitro-substituiran 1,3-diariltriazen, analog berenila® [1,3-*bis*(4'-amidinofenil)-triazena], spada v novo, kemijsko modificirano skupino učinkovitih antitumornih spojin. Njegove strukturne značilnosti smo študirali z rentgensko strukturno analizo in z ^{1}H NMR spektroskopijo, da bi ugotovili celotno konformacijo in možnost morebitne interakcije z DNA.